

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A61K 7/46, 39/12, 37/22, 53/00, A01N 37/08, C07H 17/00, 19/00, 21/00, C12P 21/06, C12N 5/00	A1	(11) International Publication Number: WO 95/03777 (43) International Publication Date: 9 February 1995 (09.02.95)
(21) International Application Number: PCT/US94/08685 (22) International Filing Date: 1 August 1994 (01.08.94) (30) Priority Data: 08/100,870 2 August 1993 (02.08.93) US (60) Parent Application or Grant (63) Related by Continuation US 08/100,870 (CIP) Filed on 2 August 1993 (02.08.93) (71) Applicant (for all designated States except US): THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10666 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): CHISARI, Francis, V. [US/US]; 1010 Crest Road, Del Mar, CA 92014 (US). (74) Agents: PARMELEE, Steven, W. et al.; Townsend and Townsend Khourie and Crew, Steuart Street Tower, 20th floor, One Market Plaza, San Francisco, CA 94105 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD). Published <i>With international search report.</i>
(54) Title: PEPTIDES FOR INDUCING CYTOTOXIC T LYMPHOCYTE RESPONSES TO HEPATITIS B VIRUS		
(57) Abstract Peptides are used to define epitopes that stimulate HLA-restricted cytotoxic T lymphocyte activity against hepatitis B virus antigens. The peptides are derived from regions of HBV polymerase, and are particularly useful in treating or preventing HBV infection, including methods for stimulating the immune response of chronically infected individuals to respond to HBV antigens.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Larvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5
PEPTIDES FOR INDUCING CYTOTOXIC T LYMPHOCYTE
RESPONSES TO HEPATITIS B VIRUS

Related Applications

10 The present application is a continuation-in-part
application of USSN 08/100,870, filed August 2, 1993, which is
a continuation-in-part of USSN 07/935,898, which is a
continuation-in-part of USSN 07/749,540, the disclosures of
which are incorporated herein by reference.

15 Government Support

The U.S. Government may have certain rights in this
invention pursuant to grants awarded by the National
Institutes of Health.

20 Background of the Invention

Cytotoxic T lymphocytes (CTLs) play an essential role
in fighting cells infected with viruses, intracellular
bacteria and parasites, and tumor cells. They do so by direct
25 cytotoxicity and by providing specific and nonspecific help to
other immunocytes such as macrophages, B cells, and other T
cells. Infected cells or tumor cells process antigen through
intracellular events involving proteases. The processed
antigen is presented on the cellular surface in the form of
30 peptides bound to HLA class I molecules to T cell receptors on
CTLs. MHC class I molecules can also bind exogenous peptides
and present them to CTLs without intracellular processing.

At the present time it is difficult to accurately
predict from the sequence of an antigenic protein how the
35 protein will be processed and which peptide portions will bind
HLA class I molecules and be presented to CTLs. Binding
motifs have been predicted for some HLA class I molecules
based on sequence analysis of peptides eluted from these
molecules (Falk et al., Nature 351:290 (1991)). Further, of

the peptides that are processed and do bind to HLA class I, which ones will contain CTL-recognizable epitopes is not yet predictable.

Hepatitis B Virus ("HBV") is a non-lytic virus which has currently infected approximately 250 million people worldwide. HBV infection in adults typically leads to an acute disease in the majority of cases, and to a chronic disease state in a minority of patients. This ratio of acute to chronic is reversed when the infection occurs close to the time of birth. There is an increased incidence of hepatocellular carcinoma in chronic HBV infection. A small percentage of individuals who are infected with HBV in adulthood develop fulminant hepatitis associated with a strong immune response with high lethality.

While there is no effective treatment for HBV infection, vaccines have been developed in recent years to prevent HBV infection. The vaccines employ either HBV surface antigen (HBsAg) purified from the plasma of chronic HBV carriers, or HBsAg produced by recombinant DNA technology. Synthetic HBsAg peptide-based vaccines have also been proposed. See, for example, U.S. Patent Nos. 4,599,230 and 4,599,231. The anti-HBsAg vaccines, however, afford protection in only about 90% of immunized individuals. Those who are unimmunized, or immunized but unprotected, provide a significant reservoir of potential infection.

The contribution of CTLs to immunity to HBV antigens has been difficult to assess. Chisari et al. (Microbial Pathogen. 6:31 (1989)) have suggested that liver cell injury may be mediated by an HLA-Class I restricted, CD8⁺ cytotoxic T cell response to HBV encoded antigens. Class I major histocompatibility (MHC) -restricted cytotoxic T lymphocyte responses have been identified for a variety of other viruses, such as influenza. For example, Townsend et al., Cell 44:959 (1986) reported that epitopes of an influenza virus nucleoprotein recognized by cytotoxic T lymphocytes could be defined by synthetic peptides. In attempting to define the cytotoxic T lymphocyte response to HBV, it has been shown that peripheral blood lymphocytes from patients with acute and

chronic HBV may be able to kill autologous hepatocytes in vitro, but the specificity of the cytolytic activity, its HLA restriction elements, and cellular phenotype were not established. See, Mondelli et al., J. Immunol. 129:2773 (1982) and Mondelli et al., Clin. Exp. Immunol. 6:311 (1987). Moriyama et al., Science 248:361-364 (1990), have reported that the HBV major envelope antigen is expressed at the hepatocyte surface in a form recognizable by envelope-specific antibodies and by MHC class I-restricted, CD8⁺ cytotoxic T lymphocytes.

As there is a large reservoir of individuals chronically infected with HBV, it would be desirable to stimulate the immune response of these individuals to respond to appropriate HBV antigens and thereby eliminate their infection. It would also be desirable to prevent the evolution to a chronic HBV infection in individuals suffering from an acute phase infection. Further, as the presently approved HBV vaccines do not elicit protective immunity in about 10% of immunized individuals, it would be desirable to elicit more effective immunity, such as by increasing or diversifying the immunogenicity of the vaccines. Quite surprisingly, the present invention fulfills these and other related needs.

Summary of the Invention

The present invention provides peptides which induce MHC class I restricted cytotoxic T lymphocyte responses against HBV antigen. The peptides of interest are derived from the sequence of the HBV polymerase protein. In certain embodiments the CTL inducing peptide will have the sequence of HBpol4-13 (Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu) [Seq ID No. 12]; HBpol61-69 (Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val) [Seq ID No. 1]; HBpol108-116 (Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg) [Seq ID No. 13]; HBpol139-147 (Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg) [Seq ID No. 14]; HBpol151-160 (His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr) [Seq ID No. 15]; HBpol152-161 (Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys) [Seq ID No. 16]; HBpol 455-463 (Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu) [Seq ID No. 2];

HBpol505-514 (Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe) [Seq ID No. 17]; HBpol551-559 (Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala) [Seq ID No. 18]; HBpol575-583 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 19]; HBpol655-663 (Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile) [Seq ID No. 20]; HBpol748-757 (Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg) [Seq ID No. 21]; HBpol758-766 (Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu) [Seq ID No. 22]; HBpol773-782 (Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val) [Seq ID No. 3]; HBpol803-811 (Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val) [Seq ID No. 4]; and HBpol816-824 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 5]; or will have a sequence substantially homologous to one of the foregoing sequences. The peptide can be optionally flanked and/or modified at one or both of the N- and C-termini, as desired. Conservative substitutions, deletions and additions may be made at non-critical residue positions within the selected peptide without substantially adversely affecting its biological activity.

In the various peptide embodiments it will be understood that the peptides can be polymerized, each to itself to form larger homopolymers, or with different peptides to form heteropolymers. In some instances peptides will be combined in a composition as an admixture and will not be linked. The peptide can also be conjugated to a lipid-containing molecules capable of enhancing a T lymphocyte response, or to a different peptide which induces a T-helper cell response, for example.

Compositions are provided which comprise a peptide of the invention formulated with an additional peptide, a liposome, an adjuvant and/or a pharmaceutically acceptable carrier. Thus, pharmaceutical compositions can be used in methods of treating acute HBV infection, particularly in an effort to prevent the infection from progressing to a chronic or carrier state. Methods for treating chronic HBV infection and HBV carrier states are also provided, where the pharmaceutical compositions are administered to infected individuals in amounts sufficient to stimulate immunogenically effective cytotoxic T cell responses against HBpol epitopes. For treating these infections it may be particularly desirable

to combine the peptides which induce MHC class I restricted cytotoxic T lymphocyte responses against HBV antigen with other peptides or proteins that induce immune response to other HBV antigens, such as HBV envelope or core. To treat individuals with chronic or carrier state infections the compositions may be administered in repeated dosages over a prolonged period of time, as necessary, to resolve or substantially mitigate the infection and/or shedding of virus.

Vaccine compositions for preventing HBV infection, particularly chronic HBV infection, are also provided. The vaccine compositions comprise an immunogenically effective amount of a HBV polymerase peptide mentioned above which induces a MHC class I restricted cytotoxic T lymphocyte response, such as HLA-A2, -A1, -A3, A-11, and/or -A24, and will typically further comprise an adjuvant, e.g., incomplete Freund's adjuvant or aluminum hydroxide. To achieve enhanced protection against HBV, the vaccine can further comprise components which elicit a protective antibody response to other HBV antigen, such as envelope (surface) antigen.

In yet other embodiments the invention relates to methods for diagnosis, where the peptides of the invention are used to determine the presence of lymphocytes in an individual which are capable of a cytotoxic T cell response to HBV polymerase antigen. The absence of such cells determines whether the individual of interest is susceptible to developing chronic HBV infection. Typically the lymphocytes are peripheral blood lymphocytes and the individual of interest is suffering from an acute HBV infection.

Brief Description of the Drawings

Fig. 1 shows the CTL response to two polymerase peptides that contain the HLA-A2 motif in a patient using target cells pulsed with peptide that match only at HLA-A2.

Fig. 2 shows the ability of several polymerase 803-811 peptide specific clones to recognize endogenously synthesized polymerase.

Fig. 3 shows that the CTL response to polymerase peptide 803-811 can recognize cells pulsed with peptide and endogenously synthesized polymerase (Vpol), whereas the CTL response to polymerase peptide 61-69 only recognized cells pulsed with the 61-69 peptide.

Fig. 4 shows the aligned amino acid sequences of 20 cloned HBV polymerase proteins; line 158 is a consensus sequence where capital letters represent 100% consensus, lower case letters represent >50% consensus, and "." is <50% consensus.

Fig. 5 shows HBV specific CTL response in patients with acute hepatitis (A-1 - A-9), chronic hepatitis (C-1 - C-9) and normal subjects (N-1 - N-9). PBMC were stimulated with the peptides indicated for 2 weeks and tested in a 4-h ^{51}Cr -release assay against JY target cells prepulsed overnight with the same peptide. Peptide-specific cytotoxicity was measured by subtracting the ^{51}Cr -release by JY target cells not prepulsed with the peptide from the ^{51}Cr -release by JY target cells prepulsed with the peptide. Results shown represent percent specific lysis in a 4 hr ^{51}Cr -release assay at an E:T of 50:1.

Fig. 6 shows CD8+ cells recognize endogenously synthesized antigen in target cells sharing the HLA-A2 allele (Patient A-1). Epitope-specific lines were generated by stimulating PBMC with the individual peptide for three weeks with weekly restimulation. On day 15 of CD4+ (positive selection) and CD8+ (negative selection) enriched lines were generated from the original bulk culture by panning. FACS-analysis showed an average enrichment by a factor of 3. Results shown represent percent specific lysis in a 4 hr ^{51}Cr -release assay at an E:T of 30:1. Targets (JY-EBV) were either

pulsed with the corresponding peptide overnight stably transfected with the polymerase expression vector.

Fig. 7 shows CTL-response to Pol455-463 GLSRVVARL [Seq ID No. 2]. Epitope-specific lines and clones, generated by stimulation with Pol455-463 peptide, were tested at varying E:T ratios against targets cells (JY-EBV), pulsed with the corresponding peptide (■) overnight or infected with recombinant vaccinia virus that express the HBV polymerase polypeptide (◆), in a standard 4 hr ⁵¹Cr-release assay. Wild-type vaccinia virus (Wt) (X) or JY-EBV peptides without peptide (O) were used as a control.

Fig. 8 shows HLA-restriction of epitope Pol455-463. Pol455-463-specific lines from patient A-1 and A-2, generated by stimulation with Pol455-463 peptide, were tested against allogeneic partly HLA-matched EBV-B cells prepulsed overnight with 10 µg/ml of the same peptide. Sharing HLA class I at other loci did not render target cells susceptible to lysis. Cytotoxicity was measured at E:T of 50:1 in a 4 hr ⁵¹Cr-release assay.

Fig. 9 shows recognition of truncated, elongated (a) or variant peptides (b) by Pol455-463 specific CTL-lines, generated by weekly stimulation of PBMC from patient A-1 with peptide Pol455-463 for 4 weeks. Cytotoxicity was measured at E:T of 50:1 in a 4 hr ⁵¹Cr-release assay against JY-EBV cells prepulsed with varying amounts of the same peptide overnight.

Description of the Specific Embodiments

The present invention provides peptides derived from HBV polymerase proteins for use in compositions and methods for the treatment, prevention and diagnosis of HBV infection. The peptides stimulate MHC HLA-class I restricted cytotoxic T lymphocyte responses against HBV infected cells. The stimulated cytotoxic T lymphocytes are able to kill the infected cells or inhibit viral replication and thus interrupt or substantially prevent infection, including chronic HBV

infection. A peptide effective in eliciting a cytotoxic T cell response may also be combined with an immunogen capable of eliciting a T-helper response.

The peptides employed in the invention are derived from the sequence of the HBV polymerase protein (HBpol), particularly CTL epitopes within HBpol4-13, HBpol61-69, HBpol108-116, HBpol139-147, HBpol151-160, HBpol152-161, HBpol455-463, HBpol505-514, HBpol551-559, HBpol575-583, HBpol655-663, HBpol748-757, HBpol758-766, HBpol773-782, HBpol803-811, or HBpol816-824, where the numbering is according to Galibert et al., supra.

By HBV cytotoxic T lymphocyte inducing "peptide" or "oligopeptide" of the present invention is meant a chain of at least four HBV amino acid sequence residues, preferably at least six, more preferably eight or nine, sometimes ten to twelve residues, and usually fewer than about fifty residues, more usually fewer than about thirty-five, and preferably fewer than twenty-five, e.g., eight to seventeen amino acid residues derived from an HBc sequence. It may be desirable to optimize peptides of the invention to a length of eight to twelve amino acid residues, more preferably nine to eleven, commensurate in size with endogenously processed viral peptides that are bound to MHC class I molecules on the cell surface. See generally, Schumacher et al., Nature 350:703-706 (1991); Van Bleek et al., Nature 348:213-216 (1990); Rotzschke et al., Nature 348:252-254 (1990); and Falk et al., Nature 351:290-296 (1991), which are incorporated herein by reference. As set forth in more detail below, usually the peptides will have at least a majority of amino acids which are homologous to a corresponding portion of contiguous residues of the HBV pol sequences herein, and contain a CTL-inducing epitope.

The peptides can be prepared "synthetically," as described hereinbelow, or by recombinant DNA technology. Although the peptide will preferably be substantially free of other naturally occurring HBV proteins and fragments thereof, in some embodiments the peptides can be synthetically conjugated to native fragments or particles. The term peptide

is used interchangeably with polypeptide in the present specification to designate a series of amino acids connected one to the other by peptide bonds between the alpha-amino and alpha-carboxy groups of adjacent amino acids. The polypeptides or peptides can be a variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the biological activity of the polypeptides as herein described.

Desirably, the peptide will be as small as possible while still maintaining substantially all of the biological activity of the large peptide. By biological activity is meant the ability to bind an appropriate MHC molecule and induce a cytotoxic T lymphocyte response against HBV antigen or antigen mimetic. By a cytotoxic T lymphocyte response is meant a CD8⁺ T lymphocyte response specific for an HBV antigen of interest, wherein CD8⁺, MHC class I-restricted T lymphocytes are activated. The activated T lymphocytes secrete lymphokines (e.g., gamma interferon) liberate products (e.g., serine esterases) that inhibit viral replication in infected autologous cells or transfected cells, with or without cell killing.

The terms "homologous", "substantially homologous", and "substantial homology" as used herein denote a sequence of amino acids having at least 50% identity wherein one sequence is compared to a reference sequence of amino acids. The percentage of sequence identity or homology is calculated by comparing one to another when aligned to corresponding portions of the reference sequence.

The peptides of the invention contain CTL-inducing epitopes derived from various epitopic regions of the HBV polymerase protein. The peptides are from the region of HBpol₆₁₋₆₉ and include peptides derived from those sequence regions which contain one or more CTL-inducing HLA class I-restricted epitopic site(s) of at least seven contiguous amino acids. A majority of the amino acids of the peptide will be

identical or substantially homologous to the amino acids of the corresponding portions of the naturally occurring HBpol₆₁₋₆₉ sequence, where HBpol₆₁₋₆₉ has the following sequence (for HBV subtype ayw):

5 (HBpol₆₁₋₆₉) [Seq. ID No. 1]
Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val, and

The peptide embodiments of this HBpol₆₁₋₆₉ region and the other polymerase peptide regions described herein can be optionally
10 flanked and/or modified at one or both of the N- and C-termini, as desired, by amino acids from HBV sequences, including HBpol, amino acids added to facilitate linking, other N- and C-terminal modifications, linked to carriers, etc., as further described herein. The peptide HBpol₆₁₋₆₉
15 induces a cytotoxic T lymphocyte response which is mediated by at least the MHC class I molecule HLA-A2.

Other HBpol region peptides containing CTL epitopes of the invention comprises the peptide HBpol 455-463, and peptides derived from HBpol₄₅₅₋₄₆₃ which contain a CTL-
20 inducing HLA class I-restricted epitopic site(s) of at least seven contiguous amino acids. A majority of the amino acids of the peptide will be identical or substantially homologous to the amino acids of the corresponding portions of the naturally occurring HBpol₄₅₅₋₄₆₃ sequence, where HBpol 455-463
25 has the sequence (for HBV subtype ayw):

(HBpol 455-463) [Seq ID No. 2]
Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu

wherein the selected peptide can be flanked and/or modified at
30 one or both termini as described herein. The peptide HBpol 455-463 induces a cytotoxic T lymphocyte response which is mediated by at least the MHC class I molecule HLA-A2.

Yet other HBpol region peptides containing CTL epitopes of the invention comprises the peptide HBpol 773-782,
35 and peptides derived from HBpol₇₇₃₋₇₈₂ which contain a CTL-inducing HLA class I-restricted epitopic site(s) of at least seven contiguous amino acids. A majority of the amino acids of the peptide will be identical or substantially homologous

to the amino acids of the corresponding portions of the naturally occurring HBpol773-782 sequence, where HBpol 773-782 has the sequence (for HBV subtype ayw):

5 (HBpol 773-782) [Seq ID No. 3]
Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val

wherein the selected peptide can be flanked and/or modified at one or both termini as described herein. The peptide HBpol
10 773-782 induces a cytotoxic T lymphocyte response which is mediated by at least the MHC class I molecule HLA-A2.
Other HBpol peptide embodiments of the invention are prepared from the region of HBpol803-811. Peptides derived from this region contain at least one CTL-inducing HLA class I-
15 restricted epitopic site, and will typically be at least seven amino acids, more usually nine, ten or eleven amino acids or more. A majority of the amino acids of the peptide will be identical or substantially homologous to the amino acids of the corresponding portions of the naturally occurring
20 HBpol803-811 sequence, where HBpol803-811 has the sequence (for HBV subtype ayw):

(HBpol₈₀₃₋₈₁₁) [Seq ID No. 4]
Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val,
25

wherein the selected peptide can be flanked and/or modified at one or both termini as described herein. The peptide HBpol
803-811 induces a cytotoxic T lymphocyte response which is mediated by at least the MHC class I molecule HLA-A2.
30 Other HBpol peptide embodiments of the invention are prepared from the region of HBpol816-824. Peptides derived from this region contain at least one CTL-inducing HLA class I-restricted epitopic site, and will typically be at least seven amino acids, more usually nine, ten or eleven amino
35 acids or more. A majority of the amino acids of the peptide will be identical or substantially homologous to the amino acids of the corresponding portions of the naturally occurring

HBpol816-824 sequence, where HBpol816-824 has the sequence
(for HBV subtype ayw):

(HBpol₈₁₆₋₈₂₄) [Seq ID No. 5]

5 Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu,

wherein the selected peptide can be flanked and/or modified at
one or both termini as described herein. The peptide HBpol
816-824 induces a cytotoxic T lymphocyte response which is
10 mediated by at least the MHC class I molecule HLA-A2.

Other HBpol peptide embodiments of the invention are
prepared from the regions of HBpol4-13, HBpol108-116,
HBpol139-147, HBpol151-160, HBpol152-161, HBpol505-514,
HBpol551-559, HBpol575-583, HBpol655-663, HBpol748-757, or
15 HBpol758-766. A peptide prepared from one of the
aforementioned regions contains at least one CTL-inducing HLA
class I-restricted epitopic site, and will typically be at
least seven amino acids, more usually nine, ten or eleven
amino acids or more. A majority of the amino acids of the
20 peptide will be identical or substantially homologous to the
amino acids of the corresponding portions of the naturally
occurring HBpol sequence, where the HBpol regions have the
sequences (for HBV subtype ayw):

HBpol4-13 [Seq ID No. 12]

25 Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu

HBpol108-116 [Seq ID No. 13]

Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg

30 HBpol139-147 [Seq ID No. 14]

Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg

HBpol151-160 [Seq ID No. 15]

His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr

35

HBpol152-161 [Seq ID No. 16]

Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys

HBpol505-514 [Seq ID No. 17]
Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe

5 HBpol551-559 [Seq ID No. 18]
Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala

HBpol575-583 [Seq ID No. 19]
Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu

10 HBpol655-663 [Seq ID No. 20]
Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile

HBpol748-757 [Seq ID No. 21]
15 Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg

HBpol758-766 [Seq ID No. 22]
Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu

wherein the selected peptide can be flanked and/or modified at
20 one or both termini as described herein. The peptide
HBpol151-160 induces a CTL response which is mediated by at
least the MHC class I molecule HLA-A1. The peptides HBpol551-
559 and HBpol655-663 induce a CTL response which is mediated
by at least the MHC class I molecule HLA-A2. The peptide
25 HBpol575-583 induces a CTL response which is mediated by at
least the MHC class I molecule HLA-A2.1. The peptides
HBpol108-116, HBpol139-147, HBpol152-161, and HBpol748-757
induce a CTL response which is mediated by at least the MHC
class I molecule HLA-A3 (HBpol748-757 appearing to also be
30 restricted by A24). The peptides HBpol4-13, HBpol505-514, and
HBpol758-766 induce CTL responses which are mediated by at
least the MHC class I molecule HLA-A24.

As mentioned above, additional amino acids can be
added to the termini of an oligopeptide or peptide to provide
35 for ease of linking peptides one to another, for coupling to a
carrier, support or a larger peptide, for reasons discussed
herein, or for modifying the physical or chemical properties
of the peptide or oligopeptide, and the like. Amino acids

such as tyrosine, cysteine, lysine, glutamic or aspartic acid, and the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, e.g., acetylation, or thioglycolic acid amidation, terminal-carboxy amidation, e.g., ammonia, methylamine, etc. In some instances these modifications may provide sites for linking to a support or other molecule.

It will be understood that the HBV peptides of the present invention or analogs or homologs thereof which have cytotoxic T lymphocyte stimulating activity may be modified as necessary to provide certain other desired attributes, e.g., improved pharmacological characteristics, while increasing or at least retaining substantially the biological activity of the unmodified peptide. For instance, the peptides can be modified by extending, decreasing or substituting amino acids in the peptide sequence by, e.g., the addition or deletion of amino acids on either the amino terminal or carboxy terminal end, or both, of peptides derived from the sequences disclosed herein. The peptides may be modified to substantially enhance the CTL inducing activity, such that the modified peptide analogs have CTL activity greater than a peptide of the wild-type sequence. For example, it may be desirable to increase the hydrophobicity of the N-terminal of a peptide, particularly where the second residue of the N-terminal is hydrophobic and is implicated in binding to the HLA restriction molecule. By increasing hydrophobicity at the N-terminal, the efficiency of the presentation to T cells may be increased. Peptides prepared from other disease associated antigens, particularly those containing CTL inducing epitopes for which a host may not have significant CTL activity, may be made CTL-inducing by substituting hydrophobic residues at the N-terminus of the peptide where the second residue is normally hydrophobic.

The peptides employed in the subject invention need not be identical to peptides HBpol4-13 (Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu) [Seq ID No. 12]; HBpol61-69 (Gly-Leu-Tyr-

Ser-Ser-Thr-Val-Pro-Val) [Seq ID No. 1]; HBpol108-116 (Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg) [Seq ID No. 13]; HBpol139-147 (Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg) [Seq ID No. 14]; HBpol151-160 (His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr) [Seq ID No. 15]; HBpol152-161 (Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys) [Seq ID No. 16]; HBpol 455-463 (Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu) [Seq ID No. 2]; HBpol505-514 (Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe) [Seq ID No. 17]; HBpol551-559 (Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala) [Seq ID No. 18]; HBpol575-583 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 19]; HBpol655-663 (Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile) [Seq ID No. 20]; HBpol748-757 (Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg) [Seq ID No. 21]; HBpol758-766 (Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu) [Seq ID No. 22]; HBpol773-782 (Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val) [Seq ID No. 3]; HBpol803-811 (Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val) [Seq ID No. 4]; or HBpol816-824 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 5], so long as the subject compounds are able to provide for cytotoxic T lymphocytic activity against at least one of the four major subtypes of HBV. Although different strains of HBV exist, they each share at least one common envelope determinant, which is designated "a". Each strain also has two other envelope determinants, one of which is either "d" or "y", and the second is either "w" or "r". Thus, there are four possible subtypes of the virus: adw, ayw, adr, and ayr. The cloning, sequencing and expression of HBV are described in GB 2034323, EP 13828, U.S. 4,935,235, and the complete sequence of the HBV envelope region is also described in Galibert et al., Nature 281:646 (1979), each of the foregoing being incorporated herein by reference. Amino acid sequences are described in the GenBank-72 database for 20 different HBV strains, including 7 of the adw subtype, 5 of the ayw subtype, 7 of the adr subtype, and 1 strain of the ayr subtype, the GenBank sequences also being incorporated herein by reference.

Therefore, the peptides may be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes provide for certain advantages in their use. By conservative

substitutions is meant replacing an amino acid residue with another which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Usually, the portion of the sequence which is intended to substantially mimic an HBV cytotoxic T lymphocyte stimulating epitope will not differ by more than about 20% from the sequence of at least one subtype of HBV, except where additional amino acids may be added at either terminus for the purpose of modifying the physical or chemical properties of the peptide for, e.g., ease of linking or coupling, and the like. Where regions of the peptide sequences are found to be polymorphic among HBV subtypes, it may be desirable to vary one or more particular amino acids to more effectively mimic differing cytotoxic T-lymphocyte epitopes of different HBV strains or subtypes.

Within the peptide sequences identified by the present invention, including the representative peptides listed above, there are residues (or those which are substantially functionally equivalent) which allow the peptide to retain their biological activity, i.e., the ability to stimulate a class I-restricted cytotoxic T-lymphocytic response against HBV infected cells or cells which express HBV antigen. These residues can be identified by single amino acid substitutions, deletions, or insertions. In addition, the contributions made by the side chains of the residues can be probed via a systematic scan with a specified amino acid (e.g., Ala). Peptides which tolerate multiple substitutions generally incorporate such substitutions as small, relatively neutral molecules, e.g., Ala, Gly, Pro, or similar residues. The number and types of residues which can be substituted, added or subtracted will depend on the spacing necessary between the essential epitopic points and certain conformational and functional attributes which are sought (e.g., hydrophobicity vs. hydrophilicity). If desired, increased binding affinity of peptide analogues to its MHC molecule for presentation to a cytotoxic T-lymphocyte can also

be achieved by such alterations. Generally, any spacer substitutions, additions or deletions between epitopic and/or conformationally important residues will employ amino acids or moieties chosen to avoid steric and charge interference which might disrupt binding.

Peptides which tolerate multiple substitutions while retaining the desired biological activity may also be synthesized as D-amino acid containing peptides. Such peptide may be synthesized as "inverso" or "retro-inverso" forms, that is, by replacing L-amino acids of a sequence with D-amino acids, or by reversing the sequence of the amino acids and replacing the L-amino acids with D-amino acids. As the D-peptides are substantially more resistant to peptidases, and therefore are more stable in serum and tissues compared to their L-peptide counterparts, the stability of D-peptides under physiological conditions may more than compensate for a difference in affinity compared to the corresponding L-peptide. Further, L-amino acid-containing peptides with or without substitutions can be capped with a D-amino acid to inhibit exopeptidase destruction of the antigenic peptide.

In addition to the exemplary peptides described herein, the invention provides methods for identifying other epitopic regions associated with said peptide regions capable of inducing MHC-restricted cytotoxic T lymphocyte responses against HBV. The methods comprise obtaining peripheral blood lymphocytes (PBL) from infected or uninfected individuals and exposing (stimulating) the cells with synthetic peptide or polypeptide fragments derived from a peptide region of HBpol4-13 (Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu) [Seq ID No. 12]; HBpol61-69 (Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val) [Seq ID No. 1]; HBpol108-116 (Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg) [Seq ID No. 13]; HBpol139-147 (Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg) [Seq ID No. 14]; HBpol151-160 (His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr) [Seq ID No. 15]; HBpol152-161 (Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys) [Seq ID No. 16]; HBpol 455-463 (Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu) [Seq ID No. 2]; HBpol505-514 (Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe) [Seq ID No. 17]; HBpol551-559 (Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala) [Seq ID No.

18]; HBpol1575-583 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 19]; HBpol655-663 (Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile) [Seq ID No. 20]; HBpol748-757 (Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg) [Seq ID No. 21]; HBpol758-766 (Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu) [Seq ID No. 22]; HBpol773-782 (Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val) [Seq ID No. 3]; HBpol803-811 (Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val) [Seq ID No. 4]; or HBpol816-824 (Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu) [Seq ID No. 5]. Pools of overlapping synthetic peptides, each typically about 8 to 20 residues long, preferably 9-12 residues, can be used to stimulate the cells. Active peptides can be selected from pools which induce cytotoxic T lymphocyte activity. The ability of the peptides to induce specific cytotoxic activity is determined by incubating the stimulated PBL with autologous labeled (e.g., ⁵¹Cr) target cells (such as HLA matched macrophages, T cells, fibroblasts or B lymphoblastoid cells) infected or transfected with the HBV subgenomic fragments thereof, such that the targeted antigen is synthesized endogenously by the cell (or the cell is pulsed with the peptide of interest), and measuring specific release of label.

Once a peptide having an epitopic region which stimulates a cytotoxic T lymphocyte response is identified, the MHC restriction element of the response can be determined. This involves incubating the stimulated PBL or short term lines thereof with a panel of (labeled) target cells of known HLA types which have been pulsed with the peptide of interest, or appropriate controls. The HLA allele(s) of cells in the panel which are lysed by the CTL are compared to cells not lysed, and the HLA restriction element(s) for the cytotoxic T lymphocyte response to the antigen of interest is identified.

Carbone et al., J. Exp. Med. 167:1767 (1988), have reported that stimulation with peptides may induce cytotoxic T lymphocytes with low affinity for corresponding endogenous protein, such that repetitive peptide stimulation may yield cytotoxic T lymphocytes that recognize peptide but not native antigen. As the inability of stimulated cytotoxic T lymphocytes to recognize native HBV proteins would be undesirable in the development of HBV peptide therapeutics and

vaccine compositions, methods to circumvent this potential limitation are used. A sequential restimulation of cytotoxic T cells is employed in the present invention to identify and select T cells with a higher affinity for naturally processed antigen than for a synthetic peptide. Short term cytotoxic T lymphocyte lines are established by restimulating activated PBL. Cells stimulated with peptide are restimulated with peptide and recombinant or native HBV antigen, e.g., HBpol. Cells having activity are also stimulated with an appropriate T cell mitogen, e.g., phytohemagglutinin (PHA). The restimulated cells are provided with irradiated allogeneic PBLs as an antigen nonspecific source of T cell help, and HBV antigen. To selectively expand the population of cytotoxic T lymphocytes that recognize native HBV antigen and to establish long term lines, PBL from a patient are first stimulated with peptide and recombinant or native HBV antigen, followed by restimulation with HLA-matched B lymphoblastoid cells that stably express the corresponding HBV antigen polypeptide. The cell lines are re-confirmed for the ability to recognize endogenously synthesized antigen using autologous and allogeneic B-lymphoblastoid or other cells transfected or infected with appropriate antigen.

Having identified different peptides of the invention which contribute to inducing anti-HBV cytotoxic T lymphocyte responses in one or more patients or HLA types, in some instances it may be desirable to join two or more peptides in a composition. The peptides in the composition can be identical or different, and together they should provide equivalent or greater biological activity than the parent peptide(s). For example, using the methods described herein, two or more peptides may define different or overlapping cytotoxic T lymphocyte epitopes from a particular region, e.g., the HBpol4-13 (Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu) [Seq ID No. 12]; HBpol61-69 (Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val) [Seq ID No. 1]; HBpol108-116 (Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg) [Seq ID No. 13]; HBpol139-147 (Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg) [Seq ID No. 14]; HBpol151-160 (His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr) [Seq ID No. 15]; HBpol152-161

(Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys) [Seq ID No. 16];
HBpol 455-463 (Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu) [Seq ID
No. 2]; HBpol505-514 (Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe)
[Seq ID No. 17]; HBpol551-559 (Tyr-Met-Asp-Asp-Val-Val-Leu-
5 Gly-Ala) [Seq ID No. 18]; HBpol575-583 (Phe-Leu-Leu-Ser-Leu-
Gly-Ile-His-Leu) [Seq ID No. 19]; HBpol655-663 (Ala-Leu-Met-
Pro-Leu-Tyr-Ala-Cys-Ile) [Seq ID No. 20]; HBpol748-757 (Gly-
Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg) [Seq ID No. 21];
HBpol758-766 (Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu) [Seq ID No.
10 22]; HBpol773-782 (Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val)
[Seq ID No. 3]; HBpol803-811 (Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-
Val) [Seq ID No. 4]; or HBpol816-824 (Phe-Leu-Leu-Ser-Leu-Gly-
Ile-His-Leu) [Seq ID No. 5] peptides, which peptides can be
combined in a "cocktail" to provide enhanced immunogenicity
15 for cytotoxic T lymphocyte responses. Moreover, peptides of
one region can be combined with peptides of other HBV regions,
from the same or different HBV protein, particularly when a
second or subsequent peptide has a MHC restriction element
different from the first. Other CTL-inducing HBV peptides are
20 described in co-pending application USSN 07/935,898 and
08/024,120, which are incorporated herein by reference. This
composition of peptides can be used to effectively broaden the
immunological coverage provided by therapeutic, vaccine or
diagnostic methods and compositions of the invention among a
25 diverse population. For example, the different frequencies of
HLA alleles among prevalent ethnic groups (caucasian, asian
and african blacks) are shown in Table I below. Therapeutic
or vaccine compositions of the invention may be formulated to
provide potential therapy or immunity to as high a percentage
30 of a population as possible.

TABLE I. HLA ALLELE FREQUENCIES AMONG PREVALENT ETHNIC GROUPS

	<u>HLA Allele</u>	<u>EUC</u>	<u>NAC</u>	<u>AFR</u>	<u>JPN</u>
5	A2	45.3	46.6	27.3	43.2
	A29	7.4	8.1	12.3	0.4
	A31	5.4	6.2	4.4	15.3
	A32	8.8	7.1	3	0.1
10	A33	3.3	3.4	9	13.1
	A28*	7.7	9.9	16.6	1.1

Abbreviations: EUC, European Caucasian; NAC, North American Caucasian; AFR, African blacks, JPN, Japanese.

15 *A28 represents the two alleles Aw68 and Aw69

The peptides of the invention can be combined via linkage to form polymers (multimers), or can be formulated in a composition without linkage, as an admixture. Where the same peptide is linked to itself, thereby forming a homopolymer, a plurality of repeating epitopic units are presented. When the peptides differ, e.g., a cocktail representing different HBV subtypes, different epitopes within a subtype, different HLA restriction specificities, a peptide which contains T helper epitopes, heteropolymers with repeating units are provided. In addition to covalent linkages, noncovalent linkages capable of forming intermolecular and intrastructural bonds are included.

Linkages for homo- or hetero-polymers or for coupling to carriers can be provided in a variety of ways. For example, cysteine residues can be added at both the amino- and carboxy-termini, where the peptides are covalently bonded via controlled oxidation of the cysteine residues. Also useful are a large number of heterobifunctional agents which generate a disulfide link at one functional group end and a peptide link at the other, including N-succidimidyl-3-(2-pyridyl-dithio) proprionate (SPDP). This reagent creates a disulfide linkage between itself and a cysteine residue in one protein and an amide linkage through the amino on a lysine or other free amino group in the other. A variety of such disulfide/amide forming agents are known. See, for example, Immun. Rev. 62:185 (1982), which is incorporated herein by reference. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these

thioether forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2 bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl) cyclohexane-1-carboxylic acid and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxy-2-nitro-4-sulfonic acid, sodium salt. A particularly preferred coupling agent is succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). It will be understood that linkage should not substantially interfere with either of the linked groups to function as described, e.g., as an HBV cytotoxic T cell determinant, peptide analogs, or T helper determinant.

In another aspect the peptides of the invention can be combined or coupled with other peptides which present HBV T-helper cell epitopes, i.e., epitopes which stimulate T cells that cooperate in the induction of cytotoxic T cells to HBV. The T-helper cells can be either the T-helper 1 or T-helper 2 phenotype, for example. T-helper epitopes from HBV sequences have been identified at HBc1-20, having the sequence: Met-Asp-Ile-Asp-Pro-Tyr-Lys-Glu-Phe-Gly-Ala-Thr-Val-Glu-Leu-Leu-Ser-Phe-Leu-Pro [Seq ID No. 6]. Other T-helper epitopes are provided by peptides from the region HBc50-69, having the sequence Pro-His-His-Tyr-Ala-Leu-Arg-Gln-Ala-Ile-Leu-Cys-Trp-Gly-Glu-Leu-Met-Tyr-Leu-Ala [Seq ID No. 7], and from the region of HBc100-139, including HBc100-119 having the sequence Leu-Leu-Trp-Phe-His-Ile-Ser-Cys-Leu-Thr-Phe-Gly-Arg-Glu-Thr-Val-Ile-Glu-Tyr-Leu [Seq ID No. 8] (where Ile₁₁₆ is Leu in the HBV adw subtype), HBc117-131 having the sequence Glu-Tyr-Leu-Val-Ser-Phe-Gly-Val-Trp-Ile-Arg-Thr-Pro-Pro-Ala [Seq ID No. 9], and peptide HBc120-139 having the sequence Val-Ser-Phe-Gly-Val-Trp-Ile-Arg-Thr-Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile [Seq ID No. 10]. See, Ferrari et al., J. Clin. Invest. 88:214-222 (1991), and U.S. Pat. 4,882,145, each incorporated herein by reference.

The peptides of the invention can be prepared in a wide variety of ways. Because of their relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various

automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co. (1984); Tam et al., J. Am. Chem. Soc. 105:6442 (1983); Merrifield, Science 232:341-347 (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds., Academic Press, New York, pp. 1-284 (1979), each of which is incorporated herein by reference.

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982), and Ausubel et al., (ed.) Current Protocols in Molecular Biology, John Wiley and Sons, Inc., New York (1987), and U.S. Pat. Nos. 4,237,224, 4,273,875, 4,431,739, 4,363,877 and 4,428,941, for example, whose disclosures are each incorporated herein by reference. Thus, fusion proteins which comprise one or more peptide sequences of the invention can be used to present the HBV cytotoxic T cell determinants. For example, a recombinant polymerase protein of the invention is prepared in which the HBpol amino acid sequence is altered so as to more effectively present epitopes of peptide regions described herein to stimulate a cytotoxic T lymphocyte response. By this means a polypeptide is used which incorporates several T cell epitopes.

As the coding sequence for peptides of the length contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., J. Am. Chem. Soc. 103:3185 (1981), modification can be made simply by substituting the appropriate base(s) for those encoding the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the

desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Yeast or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

The peptides of the present invention and pharmaceutical and vaccine compositions thereof are useful for administration to mammals, particularly humans, to treat and/or prevent HBV infection. As the peptides are used to stimulate cytotoxic T-lymphocyte responses to HBV infected cells, the compositions can be used to treat or prevent acute and/or chronic HBV infection.

For pharmaceutical compositions, the peptides of the invention as described above will be administered to an individual already infected with HBV. Those in the incubation phase or the acute phase of infection can be treated with the immunogenic peptides separately or in conjunction with other treatments, as appropriate. In therapeutic applications, compositions are administered to a patient in an amount sufficient to elicit an effective cytotoxic T lymphocyte response to HBV and to cure or at least partially arrest its symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range from about 1 μ g to about 2,000 mg of peptide for a 70 kg patient, with dosages of from about 10 μ g to about 100 mg of peptide being more commonly used, followed by booster dosages from about 1

μ g to about 1 mg of peptide over weeks to months, depending on a patient's CTL response, as determined by measuring HBV-specific CTL activity in PBLs obtained from the patient. It must be kept in mind that the peptides and compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the peptides, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of cytotoxic T-lymphocyte stimulatory peptides of the invention sufficient to effectively treat the patient.

For therapeutic use, administration should begin at the first sign of HBV infection or shortly after diagnosis in cases of acute infection, and continue until at least symptoms are substantially abated and for a period thereafter. In well established and chronic cases, loading doses followed by maintenance or booster doses may be required. The elicitation of an effective cytotoxic T lymphocyte response to HBV during treatment of acute hepatitis will minimize the possibility of subsequent development of chronic hepatitis, HBV carrier stage, and ensuing hepatocellular carcinoma.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals, about 90% of whom are capable of resolving the infection naturally. For those individuals susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where the susceptible individuals are identified prior to or during infection, for instance, as described herein, the composition can be targeted to them, minimizing need for administration to a larger population.

The peptide compositions can also be used for the treatment of chronic hepatitis and to stimulate the immune system of carriers to substantially reduce or even eliminate virus-infected cells. Those with chronic hepatitis can be identified as testing positive for virus from about 3-6 months after infection. As individuals may develop chronic HBV infection because of an inadequate (or absent) cytotoxic T lymphocyte response during the acute phase of their infection, it is important to provide an amount of immuno-potentiating peptide in a formulation and mode of administration sufficient to effectively stimulate a cytotoxic T cell response. Thus, for treatment of chronic hepatitis, a representative dose is in the range of about 1 μ g to 1,000 mg, preferably about 5 μ g to 100 mg for a 70 kg patient per dose. Administration should continue until at least clinical symptoms or laboratory indicators indicate that the HBV infection has been eliminated or substantially abated and for a period thereafter. Immunizing doses followed by maintenance or booster doses at established intervals, e.g., from one to four weeks, may be required, possibly for a prolonged period of time, as necessary to resolve the infection. For the treatment of chronic and carrier HBV infection it may also be desirable to combine the CTL peptides with other peptides or proteins that induce immune response to other HBV antigens.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the cytotoxic T-lymphocyte stimulatory peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or

lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

In some embodiments it may be desirable to include in the pharmaceutical composition at least one component which primes CTL. Lipids have been identified which are capable of priming CTL in vivo against viral antigens, e.g., tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P₃CSS), which can effectively prime virus specific cytotoxic T lymphocytes when covalently attached to an appropriate peptide. See, Deres et al., Nature 342:561-564 (1989), incorporated herein by reference. Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a cytotoxic T lymphocyte response to HBV. Further, as the induction of neutralizing antibodies can also be primed with P₃CSS conjugated to a peptide which displays an appropriate epitope, e.g., HBsAg epitopes, the two compositions can be combined to more effectively elicit both humoral and cell-mediated responses to HBV infection.

The concentration of cytotoxic T-lymphocyte stimulatory peptides of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 1%, usually at or at least about 10% to as much as 20 to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 100 mg of peptide. Actual methods for preparing parenterally administrable compounds will be known or apparent to those skilled in the art and are described in more detail in for example, Remington's

Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, PA (1985), which is incorporated herein by reference.

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue or HBV-infected hepatic cells. Liposomes can also be used to increase the half-life of the peptide composition. Liposomes useful in the present invention include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor, prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide of the invention can be directed to the site of lymphoid or hepatic cells, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference. For targeting to the immune cells, a ligand to be incorporated into the liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, etc. in a dose which varies according to, the mode of administration, the peptide being delivered, the stage of disease being treated, etc.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example,

pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the cytotoxic T-lymphocyte stimulatory peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included as desired, e.g., lecithin for intranasal delivery.

In another aspect the present invention is directed to vaccines which contain as an active ingredient an immunogenically effective amount of a cytotoxic T-lymphocyte stimulating peptide as described herein. The peptide(s) may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or cytotoxic T cells that react with different antigenic determinants of HBV. Useful carriers are well known in the art, and include, e.g., keyhole limpet hemocyanin, thyroglobulin, albumins such as human serum albumin, tetanus

toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art. And, as mentioned above, cytotoxic T lymphocyte responses can be primed by conjugating peptides of the invention to lipids, such as P₃CSS. Upon immunization with a peptide composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds to the vaccine by producing large amounts of cytotoxic T-lymphocytes specific for HBV antigen, and the host becomes at least partially immune to HBV infection, or resistant to developing chronic HBV infection.

Vaccine compositions containing the peptides of the invention are administered to a patient susceptible to or otherwise at risk of HBV infection to enhance the patient's own immune response capabilities. Such an amount is defined to be a "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the mode of administration, the nature of the formulation, etc., but generally range from about 1.0 µg to about 500 mg per 70 kilogram patient, more commonly from about 50 µg to about 200 mg per 70 kg of body weight. The peptides are administered to individuals of an appropriate HLA type, e.g., for vaccine compositions of peptides from the region of HBpol61-69 [Seq ID No. 1], Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val; HBpol 455-463 [Seq ID No. 2], Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu; HBpol551-559 and HBpol655-663; HBpol 773-782 [Seq ID No. 3], Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val; HBpol803-811 [Seq ID No. 4], Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val; or HBpol816-824 [Seq ID No. 5], Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu, these will be administered to at least HLA-A2 individuals. For peptides from HBpol151-160, these will be administered to at least HLA-A1 individuals. Vaccines comprising peptides from HBpol575-583 will be administered to at least HLA-A2.1 individuals. Vaccines comprising peptides

from HBpol575-583 will be administered to at least HLA-A2.1 individuals. Vaccines comprising peptides from HBpol108-116, HBpol139-147, HBpol152-161, and HBpol748-757 will be administered to at least HLA-A3 individuals, and/or A24 individuals in the case of HBpol748-757. The peptides HBpol4-13, HBpol505-514, and HBpol758-766 will be administered to at least HLA-A24 individuals.

In some instances it may be desirable to combine the peptide vaccines of the invention with vaccines which induce neutralizing antibody responses to HBV, particularly to HBV envelope and/or core antigens, such as recombinant HBV env- and/or nucleocapside-encoded antigens or vaccines prepared from purified plasma preparations obtained from HBV-infected individuals. A variety of HBV vaccine preparations have been described, and are based primarily on HBsAg and polypeptide fragments thereof. For examples of vaccines which can be formulated with the peptides of the present invention, see generally, EP 154,902 and EP 291,586, and U.S. Pat. Nos. 4,565,697, 4,624,918, 4,599,230, 4,599,231, 4,803,164, 4,882,145, 4,977,092, 5,017,558 and 5,019,386, each being incorporated herein by reference. The vaccines can be combined and administered concurrently, or as separate preparations.

For therapeutic or immunization purposes, the peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the HBV peptides of the invention. Upon introduction into an acutely or chronically HBV-infected host or into a non-infected host, the recombinant vaccinia virus expresses the HBV peptide and thereby elicits a host cytotoxic T lymphocyte response to HBV. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848, incorporated herein by reference. Another vector is BCG (bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful for therapeutic administration or

immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

The compositions and methods of the claimed invention
5 may be employed for ex vivo therapy. By ex vivo therapy is meant that therapeutic or immunogenic manipulations are performed outside the body. For example, lymphocytes or other target cells may be removed from a patient and treated with high doses of the subject peptides, providing a stimulatory
10 concentration of peptide in the cell medium far in excess of levels which could be accomplished or tolerated by the patient. Following treatment to stimulate the CTLs, the cells are returned to the host to treat the HBV infection. The host's cells may also be exposed to vectors which carry genes
15 encoding the peptides, as described above. Once transfected with the vectors, the cells may be propagated in vitro or returned to the patient. The cells which are propagated in vitro may be returned to the patient after reaching a predetermined cell density.

20 In one method, ex vivo CTL responses to a HBV are induced by incubating in tissue culture a patient's CTL precursor cells (CTLp) together with a source of antigen-presenting cells (APC) and the appropriate immunogenic peptide. After an appropriate incubation time (typically 1-4
25 weeks), in which the CTLp are activated and mature and expand into effector CTL, the cells are infused back into the patient, where they will destroy their specific target cell (an HBV infected cell). To optimize the in vitro conditions for the generation of specific cytotoxic T cells, the culture
30 of stimulator cells is typically maintained in an appropriate serum-free medium. Peripheral blood lymphocytes are conveniently isolated following simple venipuncture or leukapheresis of normal donors or patients and used as the responder cell sources of CTLp. In one embodiment, the
35 appropriate APC are incubated with about 10-100 μM of peptide in serum-free media for 4 hours under appropriate culture conditions. The peptide-loaded APC are then incubated with

the responder cell populations in vitro for 5 to 10 days under optimized culture conditions.

Positive CTL activation can be determined by assaying the cultures for the presence of CTLs that kill radiolabeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed form of the HBV polymerase antigen from which the peptide sequence was derived. Specificity and MHC restriction of the CTL of a patient can be determined by a number of methods known in the art. For instance, CTL restriction can be determined by testing against different peptide target cells expressing appropriate or inappropriate human MHC class I. The peptides that test positive in the MHC binding assays and give rise to specific CTL responses are identified as immunogenic peptides.

The induction of CTL in vitro requires the specific recognition of peptides that are bound to allele specific MHC class I molecules on APC. Peptide loading of empty major histocompatibility complex molecules on cells allows the induction of primary CTL responses. Since mutant cell lines do not exist for every human MHC allele, it may be advantageous to use a technique to remove endogenous MHC-associated peptides from the surface of APC, followed by loading the resulting empty MHC molecules with the immunogenic peptides of interest. The use of non-transformed, non-infected cells, and preferably, autologous cells of patients as APC is desirable for the design of CTL induction protocols directed towards development of ex vivo CTL therapies. Typically, prior to incubation of the APCs with the CTLp to be activated, an amount of antigenic peptide is added to the APC or stimulator cell culture, of sufficient quantity to become loaded onto the human Class I molecules to be expressed on the surface of the APCs. Resting or precursor CTLs are then incubated in culture with the appropriate APCs for a time period sufficient to activate the CTLs. Preferably, the CTLs are activated in an antigen-specific manner. The ratio of resting or precursor CTLs to APCs may vary from individual to individual and may further depend upon variables such as the amenability of an individual's

lymphocytes to culturing conditions and the nature and severity of the disease condition or other condition for which the described treatment modality is used. Preferably, however, the CTL:APC ratio is in the range of about 30:1 to 300:1. The CTL/APC may be maintained for as long a time as is necessary to stimulate a therapeutically useable or effective number of CTL.

Activated CTL may be effectively separated from the APC using one of a variety of known methods. For example, monoclonal antibodies specific for the APCs, for the peptides loaded onto the stimulator cells, or for the CTL (or a segment thereof) may be utilized to bind their appropriate complementary ligand. Antibody-tagged molecules may then be extracted from the admixture via appropriate means, e.g., via well-known immunoprecipitation or immunoassay methods.

Effective, cytotoxic amounts of the activated CTLs can vary between in vitro and in vivo uses, as well as with the amount and type of cells that are the ultimate target of these killer cells. The amount will also vary depending on the condition of the patient and should be determined via consideration of all appropriate factors by the practitioner. Preferably, however, about 1×10^6 to about 1×10^{12} , more preferably about 1×10^8 to about 1×10^{11} , and even more preferably, about 1×10^9 to about 1×10^{10} activated CD8+ cells are utilized for adult humans, compared to about 5×10^6 - 5×10^7 cells used in mice.

Methods of reintroducing cellular components are known in the art and include procedures such as those exemplified in U.S. Patent No. 4,844,893 to Honsik, et al. and U.S. Patent No. 4,690,915 to Rosenberg, which are incorporated herein by reference. For example, administration of activated CTLs via intravenous infusion is typically appropriate.

The peptides may also find use as diagnostic reagents. For example, a peptide of the invention may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an

affected individual. In addition, the peptides may also be used to predict which individuals will be at substantial risk for developing chronic HBV infection.

5 The following examples are offered by way of illustration, not by way of limitation.

EXAMPLE I

HLA-Restricted CTL Response TO HBV Polymerase Epitopes

10 This Example describes the identification of an HLA-A2 restricted CTL response to two HBV polymerase peptides in a patient with acute viral hepatitis. The epitopes are present in amino acid sequences HBpol₆₁₋₆₉ [Seq ID No. 1] Gly-Leu-Tyr-Ser-Ser-Thr-Val-Pro-Val (GLYSSTVPV) (also designated peptide 927.32) and HBpol₈₀₃₋₈₁₁ [Seq ID No. 4] Ser-Leu-Tyr-Ala-Asp-Ser-Pro-Ser-Val (SLYADSPSV) (also designated peptide 927.27).

15 The CTL induced by the HBpol peptides were identified in PBMCs from a patient with acute hepatitis according to the procedure set forth in Example VI of pending application USSN 07/935,898, except that the PMBCs were stimulated with individual peptides rather than peptide mixtures. The resulting CTL lines and/or clones were then tested for the ability to kill HLA-A2 matched target cells that were either pulsed with the peptide or that expressed the corresponding endogenous polymerase antigen (Vpol or EBO-pol). Construction of the vaccinia based Vpol and Epstein-Barr virus based EBO-pol constructs was as described in Example II of USSN 25 07/935,898.

As shown in Fig. 1, both peptides HBpol₈₀₃₋₈₁₁ and HBpol₆₁₋₆₉ stimulated CTL responses in a patient (HLA-A2⁺) using target cells pulsed with peptide, whereas other peptides 30 927.24 (WILRGTSFR) [Seq ID No. 23] and 927.30 (DLNLGNLNV) [Seq ID No. 24] and media controls did not stimulate the specific CTL response. The ability of the HBpol₈₀₃₋₈₁₁ specific clones to recognize endogenously synthesized polymerase antigen (Vpol and EBO-pol) is shown in Fig. 2. Two clones, designated 35 Be.27-1A1 and Be.27-1A5, were identified that recognized the HBpol₈₀₃₋₈₁₁ peptide. As shown in Fig. 3, CTL responses to HBpol₆₁₋₆₉ and HBpol₈₀₃₋₈₁₁ were shown with target cells pulsed

with homologous peptide, but only the HBpol₈₀₃₋₈₁₁ clone showed a response to endogenously synthesized Vpol antigen.

5

EXAMPLE II

This example demonstrates that acutely infected patients with clinically apparent viral hepatitis develop an HLA class I restricted CTL response to multiple epitopes in the conserved functional domains of the HBV polymerase protein, while persistently infected patients with chronic hepatitis and normal uninfected controls do not.

Nine HLA-A2 positive patients with acute hepatitis B, nine patients with chronic hepatitis B and ten healthy uninfected subjects were studied (Table II). The diagnosis of acute hepatitis B was based on clinical and biochemical evidence of acute liver injury according to standard diagnostic criteria, together with serological evidence of acute HBV infection, i.e., hepatitis B surface antigen (HBsAg, hepatitis B e antigen (HBeAg) and IgM anti-HBc antibody (IgM HBc-Ab), and the absence of serologic evidence of hepatitis delta or hepatitis C virus infection. Six of the nine patients recovered completely with normalization of serum transaminases and clearance of HBsAg and HBeAg within four months of initial diagnosis; the remaining three patients were lost to follow up. All patients with chronic hepatitis B were repeatedly serologic positive for HBsAg for more than six months and displayed mildly to moderately elevated serum ALT activity. Normal controls had no clinical history of HBV infection and were serologically negative for all HBV markers.

The amino acid sequence of HBV polymerase was screened for 9-mers and 10-mers containing the HLA-A2 allele specific binding motif. This search yielded 220 candidate peptides. Out of this group 44 peptides were selected based on conservation in at least 4 of the 7 HBV adw sequences in the GenBank Database. Lyophilized peptides were reconstituted at 20 mg/ml in DMSO and diluted to 1 mg/ml with RPMI 1640 medium.

Table II. Characteristics of Subjects Studied

	Subject	Sex	Diagnosis	HLA class I haplotype
5	A-1	Male	Acute	A2, A24, B51, B53, Cw1
	A-2	Male	Acute	A2, A63, B44, B54, Cw7
	A-3	Male	Acute	A2, A24, B27, B71/72, Cw1, Cw4
	A-4	Female	Acute	A2, A31, B51, B6, Cw3
	A-5	Male	Acute	A2, A30, B44, B35, Cw4, Cw7
	A-6	Female	Acute	A2, A69, B53, Cw4
10	A-7	Female	Acute	A2, A74, B62, B57, Cw3, Cw6
	A-8	Male	Acute	A2, A68, B58, B27, Cw1, Cw6
	A-9	Male	Acute	A2, A30, B35, Cw5
15	CH-1	Male	Chronic	A2, A23, B44
	CH-2	Male	Chronic	A2, A1, B8, B44, Cw7, Cw4
	CH-3	Male	Chronic	A2, A68, B59, B44, Cw5, Cw7
	CH-4	Male	Chronic	A2, B7801, B13, Cw7
	CH-5	Male	Chronic	A2, A30, B44, B13, Cw6
	CH-6	Male	Chronic	A2, A34, B8, B27, Cw7
20	CH-7	Male	Chronic	A2, A33, B62, B67, Cw8
	CH-8	Male	Chronic	A2, A69, B41, B52
	CH-9	Male	Chronic	A2, A25, B18, Cw6
25	N-1	Female	Normal	A2, A32, B18, B60, Cw3, Cw7
	N-2	Male	Normal	A2, B44, Cw7
	N-3	Male	Normal	A2, A1, B8, B18, Cw7
	N-4	Female	Normal	A2, B44, Cw63
	N-5	Male	Normal	A2, A23, B5, B58, Cw2, Cw6
	N-6	Male	Normal	A2, B35, B56, Cw1, Cw3
30	N-7	Male	Normal	A2, A11, B8, B62, Cw4, Cw7
	N-8	Female	Normal	A2, A3, B7, B60, Cw3, Cw7
	N-9	Male	Normal	A2, A11, B35, B44, Cw4
	N-10	Male	Normal	A2, A3, B13, B35, Cw4

35

40

45

The binding affinity of the peptides to the class I molecule was determined by competitive binding assays using the radiolabeled peptide FLPSDYFPSV [Seq ID No. 25] representing HBc18-27. The peptide was iodinated to a specific activity of $5-10 \times 10^7$ cpm/mol by the chloramine T method of Buus et al., Science 235: 1353 (1987), incorporated herein by reference. Purified class I molecules (10 to 50 nM) were incubated at room temp. with various doses of the peptides, together with 5 to 10 nM of the labeled peptide and 1 μ M human β 2-microglobulin in PBS, pH 7.0, 0.05% NP-40, 1 mM PMSF, 1.3 mM 1,10-phenanthroline, 73 μ M pepstatin A, 8 mM EDTA, and 200 μ M TLCK. After 48 hrs., class I-peptide

complexes were separated from free peptide by gel filtration on either a TSK2000 (7.8mm x 15cm) column eluted with PBS pH 6.5, 0.5% NP-40, 0.1% NaN₃, or a Sephadex G-50 column (22ml bed volume) eluted with the same buffer at pH 7.0. Class I-bound and free radioactivity was measured and the doses of peptides yielding 50% inhibition of the binding of the labeled peptide (IC₅₀) were calculated. Before conducting inhibition assays, purified class I molecules were titered in the presence of a fixed amount of labeled peptide to determine the concentration necessary to bind 10 to 30% of the total radioactivity added. All subsequent inhibition assays were then performed using these class I concentrations. Each peptide was tested in two to four independent experiments.

Fifteen of the peptides displayed an HLA-A2.1 binding affinity ratio greater than 0.01 (Table III), a threshold below which most peptides are not immunogenic. In addition two peptides which contain HLA-A2 restricted CTL epitopes were included for comparison, HBc18-27 and HBs335-343.

Table III. Characteristics of Peptides Tested

Peptide	Amino Acid Sequence	Seq ID No.	Frequency in HBV subtypes					Binding affinity	Acute HBV patients tested	* 51Cr Release	
			total	adw	ayw	adr	ayr			Responders	Mean Range
			(20)	(7)	(5)	(7)	(1)				
10	Pol527-53	26	19	6	5	7	1	9.6000	2	0	
	*Pol575-583	19	19	6	5	7	1	0.5200	9	6	38 16-81
	*Pol816-824	5	8	4	4	0	0	0.3500	9	3	30 25-32
	Pol502-510	27	19	6	5	7	1	0.2900	3	0	
	*Pol655-665	28	19	6	5	7	1	0.2000	5	2	19 17-21
15	*Pol551-559	18	18	5	5	7	1	0.1600	7	2	21 17-25
	Pol504-512	29	16	4	4	7	1	0.1300	2	0	
	*Pol455-463	2	11	7	4	0	0	0.1200	9	6	57 20-95
	Pol526-535	30	19	6	5	7	1	0.0710	2	0	
	Pol149-158	31	20	7	5	7	1	0.0560	3	0	
20	Pol772-780	32	16	6	5	4	1	0.0180	4	0	
	*Pol773-782	3	16	6	5	4	1	0.0160	7	3	18 16-21
	Pol765-774	33	16	6	5	4	1	0.0140	2	0	
	Pol424-432	34	18	5	5	7	1	0.0130	3	0	
	*Core18-27	35	9	5	4	0	0	1.5000	9	4	64 54-78
	*Env335-343	36	20	7	5	7		0.7200	9	6	66 21-88

To stimulate PBMC with the selected synthetic peptides and rHBcAg, PBMC from patients and normal donors were separated on Ficoll-Histopaque density gradients, washed three times in Hanks Balanced Salt Solution (HBSS), resuspended in RPMI 1640 supplemented with L-glutamine (2 mM), gentamicin (10 μ g/ml), and 10% heat-inactivated human AB serum and plated in a 24-well plate at 4×10^6 cells/well. rHBcAg (Biogen, Cambridge, MA) was added to the cell cultures at 1 μ g/ml and the synthetic peptides at 10 μ g/ml. In some of the studies with healthy uninfected blood donors rHBcAg was either omitted or replaced by 10 μ g/ml tetanus toxoid (Connaught Laboratories, Swiftwater, PA) since these individuals had not been previously exposed to HBV and did not benefit from rHBcAg-induced T cell help. On days 3 and 10, 1 ml of RPMI with 10% human AB serum and rIL-2 at 10 U/ml final concentration was added to each well. On day 7, the cultures were restimulated with peptide, rIL-2 and irradiated (3000 rad) autologous feeder cells and they were tested for cytotoxic activity on day 14. Selected cultures that displayed peptide specific cytolytic activity were separated into CD4+ and CD8+ populations by panning onto anti-CD4 coated flasks (Applied Immunosciences, Santa Clara, CA) and restimulated as described above.

CTL lines were established as described above and enriched in highly cytotoxic CD8+ CTLs by cloning at 10 and 3 cells per well in 96-well microwell plates in the presence of 0.5 μ g/ml CD3-specific monoclonal antibody (Coulter Immunology, Hialeah, FL), rIL-2 (100 U/ml) and 10^5 irradiated (3000 rad) allogeneic PBMC. HBV specific clones were established by cloning at 1 and 0.3 cells per well in the same way. Growing cultures were tested for cytotoxic activity against peptide-primed target cells on day 17 and cytotoxic lines and clones were expanded in a 24-well plate and restimulated every 7 to 10 days as described above.

For the cytotoxicity assays, target cells consisted of either 1) allogeneic HLA-matched and mismatched B-LCL (Amer. Soc. Histocompat. Immunogenetics, Boston, MA),

incubated overnight with synthetic peptides at 10 $\mu\text{g/ml}$; 2) stable B-LCL transfectants that express HBsAg or HBpolAg produced by transfection of the EBV-transformed B-LCL with a panel of EBV-based expression vectors that contain the corresponding coding regions of the ayw subtype (Guilhot et al., J. Virol. 66: 2670 (1992), incorporated herein by reference); or 3) B-LCL infected with recombinant vaccinia viruses (a recombinant vaccinia virus construct that encodes the HBV polymerase protein (Vpol) was produced by insertion of a 2766 fragment representing nucleotides 2290-1874 of the HBV genome (ayw subtype) into the Sma I site of the pSCII vector by standard techniques as described in Chakrabarti et al., Mol. Cell. Biol. 5: 3403 (1985), incorporated herein by reference. Vaccinia-infected targets were prepared by infection of 10^6 cells at 50 PFU/cell on a rocking plate at room temp. for 1 h followed by a single wash and overnight incubation at 37°C. Target cells were then labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 h and washed four times with HBSS. Cytolytic activity was determined in a standard 4-h ^{51}Cr release assay using U-bottomed 96-well plates containing 5,000 targets/well. Stimulated PBMC from patients and normal controls were performed in duplicate. Percent cytotoxicity was determined from the formula $100 \times ((\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release}))$. Maximum release was determined by lysis of targets by detergent (1% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release was <20% of maximal release in all assays. The assay was considered positive if the specific ^{51}Cr release from target cells containing antigen was $\geq 15\%$ higher than the nonspecific ^{51}Cr release from antigen nonspecific ^{51}Cr release from antigen negative target cells and the nonspecific lysis was less than 15% of maximum.

As shown in Table III, eight of the nine acutely infected patients responded to at least one of the polymerase peptides and, as can be seen from Table III, six of the peptides were recognized by at least one patient, suggesting that they represented HLA-A2 restricted epitopes. The HLA

binding ratio of 5 of 6 of these peptides was greater than 0.1, supporting a direct relationship between binding affinity and immunogenicity even among this group of high affinity peptides.

5 The HLA-A2 binding affinity of a peptide did not appear to be the only requirement for immunogenicity since the peptide (LLAQFTSAI) [Seq ID No. 26] with the highest binding affinity (9.600) did not elicit an immune response while one with a 600-times lower affinity (0.016) did. To exclude the possibility that this extremely high affinity peptide may have triggered potentially responsive CTL precursors to undergo apoptosis, PBMC were also stimulated with lower concentrations of this peptide (0.3, 1, 3 and 10 μ g/ml) without inducing a CTL response, suggesting that nonresponsiveness to this and other high affinity peptides is probably due to other mechanisms.

 The CTL responses of nine acutely infected patients who responded to one or more polymerase peptides are summarized in Fig. 5. Five of these patients also recognized the two control peptides, HBc18-27 and HBenv335-343, while one patient recognized only HBenv335-343, and one patient responded to neither. These results demonstrate the clonality and multispecificity of the CTL response against the polymerase protein during acute viral hepatitis. Importantly, nine of the 10 uninfected controls responded to any of the peptides used in this example (nine of these controls are shown in Fig. 5), suggesting that the CTL responses observed in the acutely infected patients represented in vitro secondary responses that were primed by exposure to infected cells in vivo. None of the nine patients with chronic hepatitis produced a response, suggesting that the vigor of the HBV specific CTL response has a role in determining which patients will clear the virus and which patients will not.

 Having identified two HLA-A2 patients (A-1 and A-2) with acute hepatitis who responded strongly to HBpol575-583 and HBpol455-463 and HBpol816-824 (Table III), these patients and peptides were chosen for further analysis. After two weeks of in vitro stimulation, selected cultures that

displayed peptide specific CTL responses were enriched for CD4+ and CD8+ subsets by panning using positive and negative selection, respectively, and they were restimulated with peptide and tested for recognition of endogenously processed polymerase antigen after one additional week of culture. As shown in Fig. 6, the CTL response to these epitopes was mediated by CD8+ T cells since only the CD8+ fraction of each cell line recognized target cells that were either pulsed with the corresponding peptide or stably transfected with the polymerase expression vector. These results suggest that the peptides represent the native epitopes that are produced by the cellular processing of the polymerase protein, and that they are presented in the context of class I HLA molecules.

To obtain pure CD8+ cell lines and to characterize the T cell response at the clonal level, each of the three responding cell lines was cloned by limiting dilution in the presence of anti-CD3, irradiated allogeneic PBL and IL-2. All of the derivative cytotoxic lines were highly enriched in CD8+ cells as determined by FACS analysis ($0.5 - 1.0 \times 10^6$ cells were washed once in PBS with 5% BSA and 0.02% sodium azide, the pelleted cells were then stained with a fluorescent probe-conjugated anti-CD4 and anti-CD8 monoclonal antibody (Leu3a or Leu2a), and similarly labeled control antibody for 30 min. at 4°C, and after 3 washes in PBS with 5% BSA and 0.02% sodium azide, cells were analyzed with a FACScan flow cytometer). Furthermore, 5 of the 6 HBpol455-463 specific CTL clones derived in this manner also consisted of CD8+ cells.

Four highly cytotoxic long term CTL lines and two clones specific for HBpol455-463 pulsed targets were chosen for further analysis (Fig. 7). The strength of the cytotoxic activity was assessed by varying the amount of the peptide used to pulse the target cells and by varying the effector to target ratios. The CTL displayed peptide dose dependent cytotoxic activity that recognized targets pulsed with peptide concentrations as low as 10 nM (Table IV), and they efficiently lysed both peptide pulsed and vaccinia-pol infected targets at E:T ratios as low as 1.6:1 (Fig. 7). Target cells pulsed with no peptide or with an irrelevant

peptide (Table V), which is an HLA-A2 restricted epitope in HCV-infected patients, were not lysed, now were cells infected by the control recombinant vaccinia virus that expresses the HBV envelope protein, indicating the specificity of the CTL.

5

Table IV. Recognition GLSRYVARL-pulsed JY-EBV by CTL is peptide-dose dependent [Seq ID No. 2]

10

Patient	Line	JY-EBV pulsed with				no peptide
		10 μ M GLSRYVARL	1 μ M GLSRYVARL	0.1 μ M GLSRYVARL	0.01 μ M GLSRYVARL	
A-1	67-68	41%	26%	19%	13%	5%
A-2	10	75%	56%	52%	25%	12%
A-2	30	69%	40%	40%	19%	6%

15

20

Table V. Induction of CTL with variant peptides to GLSRYVARL [Seq ID No. 2] and an HCV epitope KLVALGINAV [Seq ID No. 37]

25

Peptide During CTL-Induction	% Specific Cytotoxicity Against JY-EBV Preincubated with				
	GLSRYVARL Seq ID 2	GLPRYVARL Seq ID 38	SGLSRYVARL Seq ID 39	GLSRYVARLS Seq ID 40	KLVALGINAV Seq ID 37
GLSRYVARL	54%	18%	40%	41%	2%
GLPRYVARL	1%	0%	1%	0%	
SGLSRYVARL	0%	0%	0%	0%	
GLSRYVARLS	1%	1%	0%	0%	

30

35

40

To identify the restriction element used by the HBpol455-463 specific CTL, cytotoxic lines and clones from patients A-1 and A-2 were tested against allogeneic EBV-B cell lines sharing individual HLA class I alleles with the effector cells. As shown in Fig. 8, not only was HLA-A2 the sole class I allele shared by these two patients, but their CTL only lyse peptide pulsed target cells that share this allele. Thus, HBpol455-463 specific CTLs from both patients are HLA-A2 restricted.

45

Peptides containing carboxy- and amino-terminal truncations and elongations of the HBpol455-463 sequence were synthesized to determine the optimal length and the precise termini of the epitope. As shown in Fig. 9A, truncation of Gly455 or Leu463 greatly reduced the HLA binding affinity of the peptides and totally abrogated their recognition by CTL induced by the original peptide HBpol455-463. Elongation of this peptide by adding a single Ser residue normally present upstream of the amino terminus or downstream of the carboxy-terminus of HBpol455-463 did not diminish its recognition by CTL (Fig. 9A), and may have even increased recognition, despite the fact that the HLA-A2 binding affinity of the extended peptides was reduced 4-10 fold relative to the original peptide (Fig. 9A). The Ser-extended peptides did not induce CTL, as shown in Table V.

Direct sequencing of the PCR products amplified from the serum of 5 of the 9 patients with acute hepatitis B by nested PCR demonstrated that the deduced HBV amino acid sequence was identical to GLSRYVARL [Seq ID No. 2] in these patients. The sequence is present in 7/7 and 4/5 adw and ayw subtype sequences in GenBank. The amino acid sequence of the remaining ayw isolate in the database is GVSRYVARL [Seq ID No. 41], while the sequence of 6/7 adr and 1/1 ayr isolates is GLPRYVARL [Seq ID No. 42] and the sequence of the remaining adr isolate is GLPRYVVCL [Seq ID No. 43].

Peptides containing sequences of these different viral subtypes were tested for recognition by GLSRYVARL-stimulated [Seq ID No. 2] PBMC to assess cross-reactivity of the CTL response. None of the variants was efficiently recognized by the CTL. GLSRYVVCL [Seq ID No. 44] was not recognized, even at very high peptide concentration, despite the fact that its HLA-A2.1 binding affinity was greater than the prototype peptide GLSRYVARL [Seq ID No. 2]. Thus, Ser457, Ala461 and Arg462 may represent T cell receptor contact sites (epitope residues) in this peptide. A substitution in Ser457 in GLPRYVARL [Seq ID No. 42] variant yielded more than a 10-fold reduction in its recognition by the CTL, while decreasing the HLA binding affinity 2-fold.

The GVSRYVARL [Seq ID No. 41] variant which contained the substitution at Leu456, a presumptive HLA contact site (agretope residue), was poorly recognized by the CTL, commensurate with the 9-fold reduction in its HLA-A2 binding affinity. However, the amino- and carboxy-terminally extended peptides described above were well recognized by the CTL despite the fact that they displayed comparably reduced HLA-A2 binding affinities (Fig. 9A). This suggests that Leu456 not only serves as an agretope residue, but may also influence the T cell receptor binding affinity of the peptide.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) NAME: The Scripps Research Institute
 - (B) STREET: 10666 North Torrey Pines Road
 - (C) CITY: La Jolla
 - (D) STATE: California
 - (E) COUNTRY: United States of America
 - (F) POSTAL CODE (ZIP): 92037
 - (G) TELEPHONE: (206) 467-9600
 - (H) TELEFAX: (415) 543-5043
 - (I) TELEX:
- (ii) TITLE OF INVENTION: PEPTIDES FOR INDUCING CYTOTOXIC T LYMPHOCYTE RESPONSES TO HEPATITIS B VIRUS
- (iii) NUMBER OF SEQUENCES: 44
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (v) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE: 01-AUG-1994
 - (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/100,870
 - (B) FILING DATE: 02-AUG-1993
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/935,898
 - (B) FILING DATE: 26-AUG-1992
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/749,540
 - (B) FILING DATE: 26-AUG-1991
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Parmelee, Steven W.
 - (B) REGISTRATION NUMBER: 31,990
 - (C) REFERENCE/DOCKET NUMBER: 14740-2-2
- (viii) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (206) 467-9600
 - (B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Leu Tyr Ser Ser Thr Val Pro Val

1 5

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Leu Ser Arg Tyr Val Ala Arg Leu
1 5

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ile Leu Arg Gly Thr Ser Phe Val Tyr Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Leu Tyr Ala Asp Ser Pro Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Phe Leu Leu Ser Leu Gly Ile His Leu
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
1 5 10 15
Ser Phe Leu Pro
 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Pro His His Tyr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu
1 5 10 15
Met Tyr Leu Ala
 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
1 5 10 15
Ile Glu Tyr Leu
 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Glu	Tyr	Leu	Val	Ser	Phe	Gly	Val	Trp	Ile	Arg	Thr	Pro	Pro	Ala
1				5				10						15

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val	Ser	Phe	Gly	Val	Trp	Ile	Arg	Thr	Pro	Pro	Ala	Tyr	Arg	Pro	Pro
1				5				10						15	
Asn	Ala	Pro	Ile												
			20												

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 845 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Pro	Leu	Ser	Tyr	Gln	His	Phe	Arg	Lys	Leu	Leu	Leu	Leu	Asp	Asp
1				5					10					15	
Gly	Thr	Glu	Ala	Gly	Pro	Leu	Glu	Glu	Glu	Leu	Pro	Arg	Leu	Ala	Asp
			20					25					30		
Glu	Gly	Leu	Asn	Arg	Arg	Val	Ala	Glu	Asp	Leu	Asn	Leu	Gly	Asn	Leu
		35					40					45			
Asn	Val	Ser	Ile	Pro	Trp	Thr	His	Lys	Val	Gly	Asn	Phe	Thr	Gly	Leu
	50					55					60				
Tyr	Ser	Ser	Thr	Val	Pro	Val	Phe	Asn	Pro	Glu	Trp	Gln	Thr	Pro	Ser
65				70						75				80	
Phe	Pro	Asn	Ile	His	Leu	Gln	Glu	Asp	Ile	Ile	Asn	Arg	Cys	Gln	Gln
			85						90					95	
Phe	Val	Gly	Pro	Leu	Thr	Val	Asn	Glu	Lys	Arg	Arg	Leu	Lys	Leu	Ile
			100					105					110		
Met	Pro	Ala	Arg	Phe	Tyr	Pro	Asn	Val	Thr	Lys	Tyr	Leu	Pro	Leu	Asp
		115					120					125			
Lys	Gly	Ile	Lys	Pro	Tyr	Tyr	Pro	Glu	His	Leu	Val	Asn	His	Tyr	Phe
	130					135					140				
Gln	Thr	Arg	His	Tyr	Leu	His	Thr	Leu	Trp	Lys	Ala	Gly	Ile	Leu	Tyr

145					150						155				160
Lys	Arg	Glu	Thr	Thr	Arg	Ser	Ala	Ser	Phe	Cys	Gly	Ser	Pro	Tyr	Ser
				165					170					175	
Trp	Glu	Gln	Glu	Leu	Gln	His	Gly	Arg	Leu	Val	Phe	Gln	Thr	Ser	Thr
			180					185					190		
Arg	His	Gly	Asp	Glu	Ser	Phe	Cys	Ser	Gln	Ser	Ser	Gly	Ile	Leu	Ser
		195					200					205			
Arg	Ser	Pro	Val	Gly	Pro	Cys	Ile	Arg	Ser	Gln	Leu	Arg	Gln	Ser	Arg
	210					215					220				
Leu	Gly	Leu	Gln	Pro	Gln	Gln	Gly	His	Leu	Ala	Arg	Arg	Gln	Gln	Gly
225					230					235					240
Arg	Ser	Gly	Ser	Ile	Arg	Ala	Arg	Val	His	Pro	Thr	Thr	Arg	Arg	Ser
				245					250					255	
Phe	Gly	Val	Glu	Pro	Ser	Gly	Ser	Gly	His	Ile	Asp	Asn	Ser	Ala	Ser
			260					265					270		
Ser	Ser	Ser	Ser	Cys	Leu	His	Gln	Ser	Ala	Val	Arg	Lys	Ala	Ala	Tyr
		275					280					285			
Ser	His	Leu	Ser	Thr	Ser	Lys	Arg	Gln	Ser	Ser	Ser	Gly	His	Ala	Val
	290					295					300				
Glu	Leu	His	Asn	Ile	Pro	Pro	Asn	Ser	Ala	Arg	Ser	Gln	Ser	Glu	Gly
305					310					315					320
Pro	Val	Phe	Ser	Cys	Trp	Trp	Leu	Gln	Phe	Arg	Asn	Ser	Lys	Pro	Cys
				325					330					335	
Ser	Asp	Tyr	Cys	Leu	Thr	His	Ile	Val	Asn	Leu	Leu	Glu	Asp	Trp	Gly
			340					345					350		
Pro	Cys	Thr	Glu	His	Gly	Glu	His	Asn	Ile	Arg	Ile	Pro	Arg	Thr	Pro
		355					360					365			
Ala	Arg	Val	Thr	Gly	Gly	Val	Phe	Leu	Val	Asp	Lys	Asn	Pro	His	Asn
	370					375					380				
Thr	Thr	Glu	Ser	Arg	Leu	Val	Val	Asp	Phe	Ser	Gln	Phe	Ser	Arg	Gly
385					390					395					400
Ser	Thr	Arg	Val	Ser	Trp	Pro	Lys	Phe	Ala	Val	Pro	Asn	Leu	Gln	Ser
			405						410					415	
Leu	Thr	Asn	Leu	Leu	Ser	Ser	Asn	Leu	Ser	Trp	Leu	Ser	Leu	Asp	Val
			420					425					430		
Ser	Ala	Ala	Phe	Tyr	His	Ile	Pro	Leu	His	Pro	Ala	Ala	Met	Pro	His
		435					440					445			
Leu	Leu	Val	Gly	Ser	Ser	Gly	Leu	Ser	Arg	Tyr	Val	Ala	Arg	Leu	Ser
	450					455					460				
Ser	Asn	Ser	Arg	Ile	Ile	Asn	Tyr	Gln	His	Gly	Thr	Met	Gln	Asn	Leu
465					470					475					480
His	Asp	Ser	Cys	Ser	Arg	Asn	Leu	Tyr	Val	Ser	Leu	Leu	Leu	Leu	Tyr
			485						490					495	
Lys	Thr	Phe	Gly	Arg	Lys	Leu	His	Leu	Tyr	Ser	His	Pro	Ile	Ile	Leu
			500					505					510		
Gly	Phe	Arg	Lys	Ile	Pro	Met	Gly	Val	Gly	Leu	Ser	Pro	Phe	Leu	Leu

52

515					520					525					
Ala	Gln	Phe	Thr	Ser	Ala	Ile	Cys	Ser	Val	Val	Arg	Arg	Ala	Phe	Pro
530					535					540					
His	Cys	Leu	Ala	Phe	Ser	Tyr	Met	Asp	Asp	Val	Val	Leu	Gly	Ala	Lys
545					550					555					560
Ser	Val	Gln	His	Leu	Glu	Ser	Leu	Phe	Thr	Ala	Val	Thr	Asn	Phe	Leu
				565					570					575	
Leu	Ser	Leu	Gly	Ile	His	Leu	Asn	Pro	Asn	Lys	Thr	Lys	Arg	Trp	Gly
			580					585					590		
Tyr	Ser	Leu	Asn	Phe	Met	Gly	Tyr	Val	Ile	Gly	Ser	Trp	Gly	Thr	Ile
		595					600					605			
Pro	Gln	Glu	His	Ile	Val	Gln	Lys	Ile	Lys	Gln	Cys	Phe	Arg	Lys	Leu
	610					615					620				
Pro	Val	Asn	Arg	Pro	Ile	Asp	Trp	Lys	Val	Cys	Gln	Arg	Ile	Val	Gly
625					630					635					640
Leu	Leu	Gly	Phe	Ala	Ala	Pro	Phe	Thr	Gln	Cys	Gly	Tyr	Pro	Ala	Leu
				645					650					655	
Met	Pro	Leu	Tyr	Ala	Cys	Ile	Gln	Ser	Lys	Gln	Ala	Phe	Thr	Phe	Ser
			660					665					670		
Pro	Thr	Tyr	Lys	Ala	Phe	Leu	Cys	Lys	Gln	Tyr	Leu	Asn	Leu	Tyr	Pro
		675					680					685			
Val	Ala	Arg	Gln	Arg	Pro	Gly	Leu	Cys	Gln	Val	Phe	Ala	Asp	Ala	Thr
	690					695					700				
Pro	Thr	Gly	Trp	Gly	Leu	Ala	Ile	Gly	His	Gln	Arg	Met	Arg	Gly	Thr
705					710					715					720
Phe	Val	Ala	Pro	Leu	Pro	Ile	His	Thr	Ala	Glu	Leu	Leu	Ala	Ala	Cys
				725					730					735	
Phe	Ala	Arg	Ser	Arg	Ser	Gly	Ala	Lys	Leu	Ile	Gly	Thr	Asp	Asn	Ser
			740					745					750		
Val	Val	Leu	Ser	Arg	Lys	Tyr	Thr	Ser	Phe	Pro	Trp	Leu	Leu	Gly	Cys
		755					760					765			
Ala	Ala	Asn	Trp	Ile	Leu	Arg	Gly	Thr	Ser	Phe	Val	Tyr	Val	Pro	Ser
	770					775					780				
Ala	Leu	Asn	Pro	Ala	Asp	Asp	Pro	Ser	Arg	Gly	Arg	Leu	Gly	Leu	Tyr
785					790					795					800
Arg	Pro	Leu	Leu	Arg	Leu	Pro	Phe	Arg	Pro	Thr	Thr	Gly	Arg	Thr	Ser
				805					810					815	
Leu	Tyr	Ala	Val	Ser	Pro	Ser	Val	Pro	Ser	His	Leu	Pro	Asp	Arg	Val
			820					825					830		
His	Phe	Ala	Ser	Pro	Leu	His	Val	Ala	Trp	Arg	Pro	Pro			
		835					840					845			

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Tyr Gln His Phe Arg Lys Leu Leu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg Leu Lys Leu Ile Met Pro Ala Arg
1 5

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val Val Asn His Tyr Phe Gln Thr Arg
1 5

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Thr Leu Trp Lys Ala Gly Ile Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Tyr Met Asp Asp Val Val Leu Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Phe Leu Leu Ser Leu Gly Ile His Leu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Leu Met Pro Leu Tyr Ala Cys Ile
1 5

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Thr Asp Asn Ser Val Val Leu Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Tyr Thr Ser Phe Pro Trp Leu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Trp Ile Leu Arg Gly Thr Ser Phe Arg
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asp Leu Asn Leu Gly Asn Leu Asn Val
1 5

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Phe Leu Pro Ser Asp Tyr Phe Pro Ser Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Leu Leu Ala Gln Phe Thr Ser Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Lys Leu His Leu Tyr Ser His Pro Ile
1 5

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Leu Met Pro Leu Tyr Ala Cys Ile

1 5

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

His Leu Tyr Ser His Pro Ile Ile Leu
1 5

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Leu His Thr Leu Trp Lys Ala Gly Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Trp Ile Leu Arg Gly Thr Ser Phe Val
1 5

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Leu Leu Gly Cys Ala Ala Asn Trp Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Asn Leu Ser Trp Leu Ser Leu Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Phe Leu Pro Ser Asp Phe Pro Pro Ser Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Trp Leu Ser Leu Leu Val Pro Phe Val
1 5

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Lys Leu Val Ala Leu Gly Ile Asn Ala Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Leu Pro Arg Tyr Val Ala Arg Leu
1 5

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:

60

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Val Ser Arg Tyr Val Ala Arg Leu
1 5

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gly Leu Pro Arg Tyr Val Ala Arg Leu
1 5

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Leu Pro Arg Tyr Val Val Cys Leu
1 5

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Leu Ser Arg Tyr Val Val Cys Leu
1 5

WHAT IS CLAIMED IS:

1. A CTL inducing peptide comprising from eight to thirteen amino acids, wherein at least a majority of the amino acids are homologous to a corresponding portion of HBpol
5 having the sequence:

HBpol4-13 [Seq ID No. 12]

Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu;

10 HBpol108-116 [Seq ID No. 13]

Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg;

HBpol139-147 [Seq ID No. 14]

Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg

15

HBpol151-160 [Seq ID No. 15]

His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr

HBpol152-161 [Seq ID No. 16]

20

Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys

HBpol 455-463 [Seq ID No. 2]

Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu;

25

HBpol505-514 [Seq ID No. 17]

Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe;

HBpol551-559 [Seq ID No. 18]

Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala;

30

HBpol575-583 [Seq ID No. 19]

Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu;

HBpol655-663 [Seq ID No. 20]

35

Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile;

HBpol748-757 [Seq ID No. 21]

Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg;

HBpol758-766 [Seq ID No. 22]
Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu);

5 HBpol773-782 [Seq ID No. 3]
Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val or

HBpol816-824 [Seq ID No. 5]
Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu

10

2. The CTL inducing peptide of claim 1, which
comprises from nine to eleven amino acids.

15 3. The CTL inducing peptide of claim 1, which
comprises:

HBpol4-13 [Seq ID No. 12]
Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu;

20 HBpol108-116 [Seq ID No. 13]
Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg;

HBpol139-147 [Seq ID No. 14]
Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg

25

HBpol151-160 [Seq ID No. 15]
His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr

HBpol152-161 [Seq ID No. 16]
30 Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys

HBpol 455-463 [Seq ID No. 2]
Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu;

35 HBpol505-514 [Seq ID No. 17]
Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe;

HBpol551-559 [Seq ID No. 18]

Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala;

HBpol575-583 [Seq ID No. 19]

Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu;

5

HBpol655-663 [Seq ID No. 20]

Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile;

HBpol748-757 [Seq ID No. 21]

10

Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg;

HBpol758-766 [Seq ID No. 22]

Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu);

15

HBpol773-782 [Seq ID No. 3]

Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val or

HBpol816-824 [Seq ID No. 5]

Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu

20

4. The peptide of claim 1, suspended in pharmaceutically acceptable carrier which comprises a liposome.

25

5. The CTL inducing peptide of claim 1, wherein the peptide also contains a T helper epitope.

6. The peptide of claim 1, conjugated to a immunogenic lipid carrier.

30

7. A method for stimulating a cytotoxic T lymphocyte response to hepatitis B virus which comprises exposing cytotoxic T lymphocytes of a host to a peptide which contains a CTL epitope and which comprises from eight to thirteen amino acids, wherein at least a majority of the amino acids are homologous to a corresponding portion of HBpol having the sequence:

35

HBpol4-13 [Seq ID No. 12]

Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu;

HBpol108-116 [Seq ID No. 13]

5 Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg;

HBpol139-147 [Seq ID No. 14]

Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg

HBpol151-160 [Seq ID No. 15]

10 His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr

HBpol152-161 [Seq ID No. 16]

15 Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys

HBpol 455-463 [Seq ID No. 2]

Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu;

HBpol505-514 [Seq ID No. 17]

20 Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe;

HBpol551-559 [Seq ID No. 18]

Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala;

HBpol575-583 [Seq ID No. 19]

25 Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu;

HBpol655-663 [Seq ID No. 20]

30 Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile;

HBpol748-757 [Seq ID No. 21]

Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg;

HBpol758-766 [Seq ID No. 22]

35 Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu);

HBpol773-782 [Seq ID No. 3]

Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val or

HBpol816-824 [Seq ID No. 5]
Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu

5 8. The method of claim 7, wherein the CTL inducing peptide is:

 HBpol4-13 [Seq ID No. 12]
Ser-Tyr-Gln-His-Phe-Arg-Lys-Leu-Leu-Leu;

10 HBpol108-116 [Seq ID No. 13]
Arg-Leu-Lys-Leu-Ile-Met-Pro-Ala-Arg;

 HBpol139-147 [Seq ID No. 14]
Val-Val-Asn-His-Tyr-Phe-Gln-Thr-Arg

15 HBpol151-160 [Seq ID No. 15]
His-Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr

 HBpol152-161 [Seq ID No. 16]
20 Thr-Leu-Trp-Lys-Ala-Gly-Ile-Leu-Tyr-Lys

 HBpol 455-463 [Seq ID No. 2]
Gly-Leu-Ser-Arg-Tyr-Val-Ala-Arg-Leu;

25 HBpol505-514 [Seq ID No. 17]
Leu-Tyr-Ser-His-Pro-Ile-Ile-Leu-Gly-Phe;

 HBpol551-559 [Seq ID No. 18]
Tyr-Met-Asp-Asp-Val-Val-Leu-Gly-Ala;

30 HBpol575-583 [Seq ID No. 19]
Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu;

 HBpol655-663 [Seq ID No. 20]
35 Ala-Leu-Met-Pro-Leu-Tyr-Ala-Cys-Ile;

 HBpol748-757 [Seq ID No. 21]
Gly-Thr-Asp-Asn-Ser-Val-Val-Leu-Ser-Arg;

HBpol758-766 [Seq ID No. 22]
Lys-Tyr-Thr-Ser-Phe-Pro-Trp-Leu-Leu);

5 HBpol773-782 [Seq ID No. 3]
Ile-Leu-Arg-Gly-Thr-Ser-Phe-Val-Tyr-Val or

HBpol816-824 [Seq ID No. 5]
Phe-Leu-Leu-Ser-Leu-Gly-Ile-His-Leu.

10

9. The method of claim 8, wherein the host cells exposed to the CTL-inducing peptides are HLA-A2.

15 10. The method of claim 8, wherein the cytotoxic T cells are removed from the host prior to being exposed to the CTL-inducing peptide.

20 11. The method according to claim 10, further comprising the step of returning the stimulated CTLs to the host.

12. The method of claim 8, wherein the host has chronic hepatitis B infection or is a hepatitis B carrier.

25

13. The method of claim 8, wherein the host has acute hepatitis B infection.

30 14. The method of claim 8, wherein the CTL inducing peptide is administered prophylactically to the host's cells.

15. The method of claim 8, wherein the CTL inducing peptide is administered to the host with a second peptide which elicits a T helper response to HBV.

35

16. The method of claim 15, wherein the CTL inducing peptide and the T helper inducing peptide are linked.

1/15

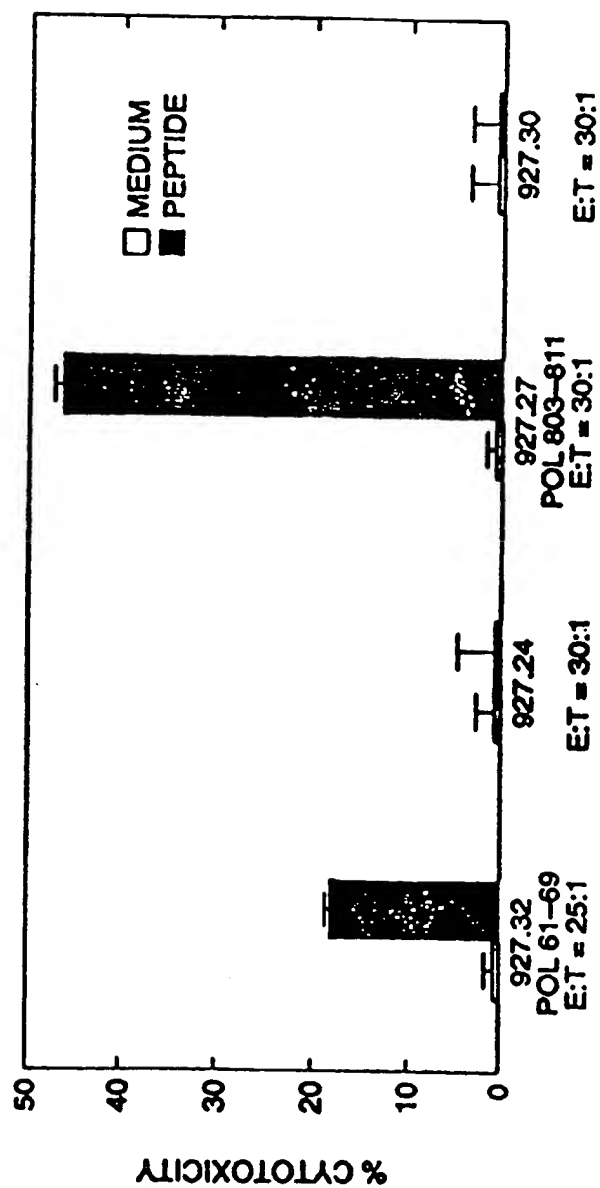


FIG. 1

2/15

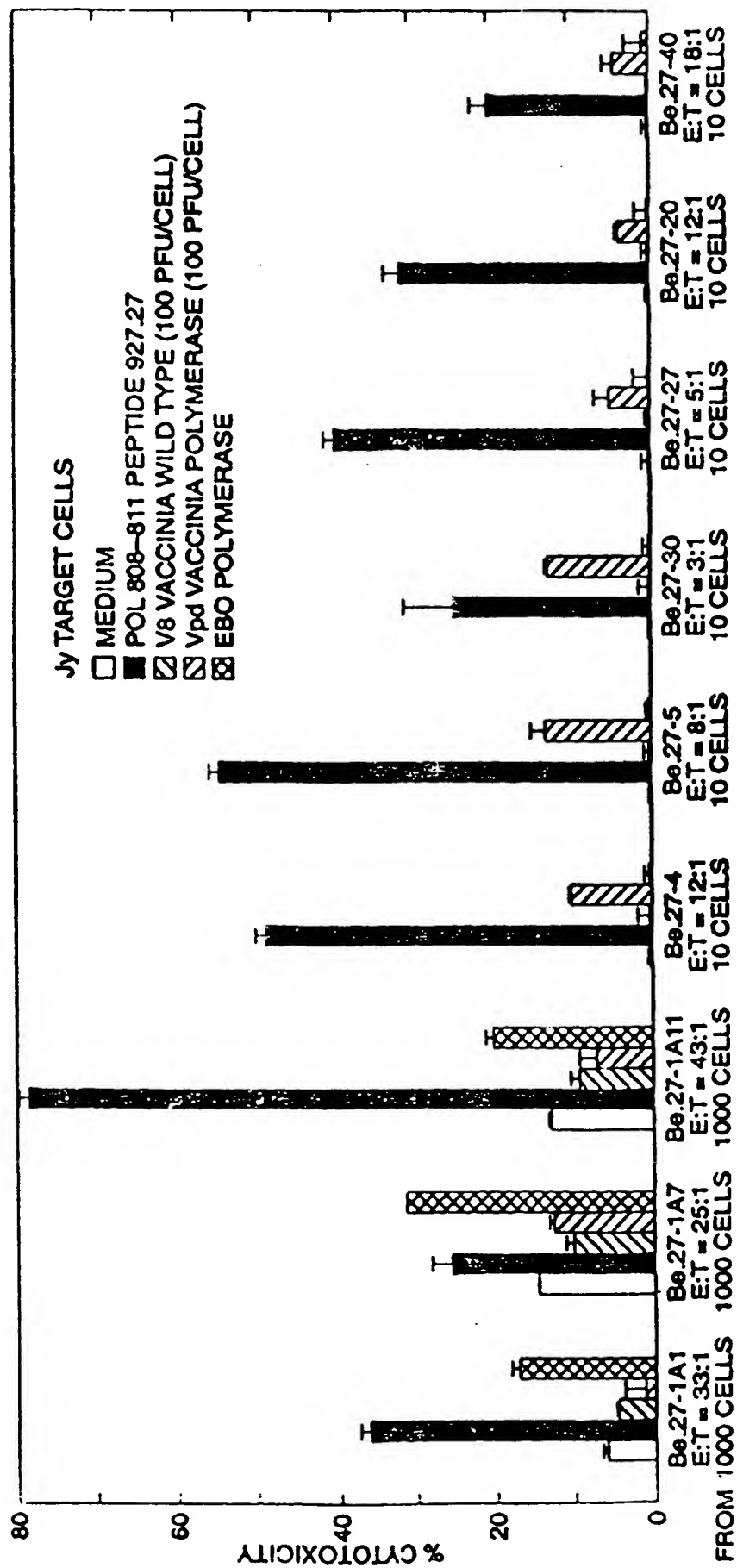


FIG. 2

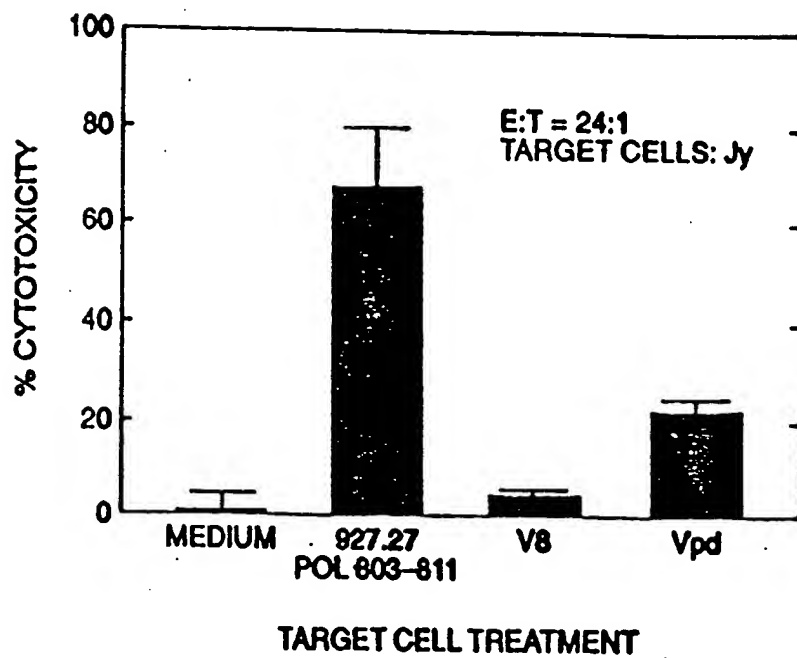


FIG. 3A

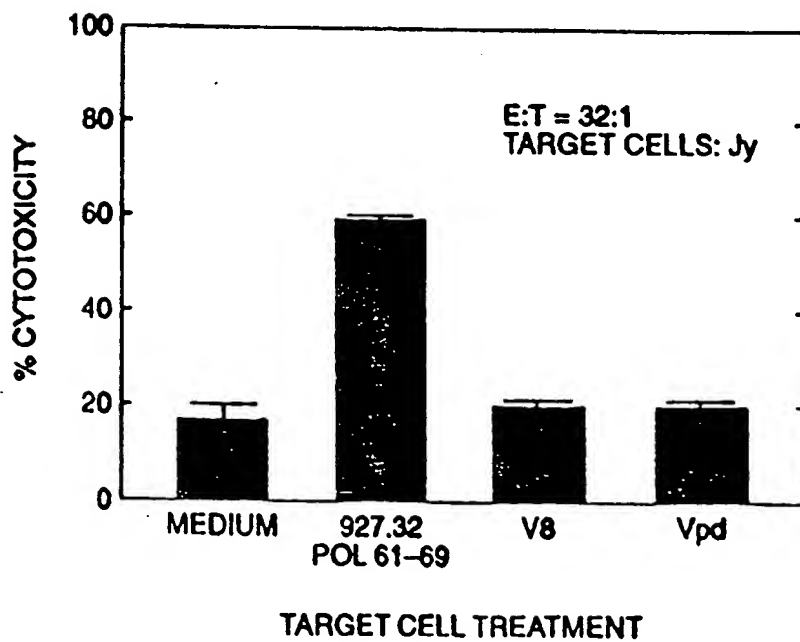


FIG. 3B

4/15

1
39 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
40 MPLSYQHFRKLLLLD???EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
41 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
42 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
43 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
44 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
45 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
46 MPLSYQHFRKLLLLDDGTEA GPLEEELPRLADADLNRRVA EDNLGNLNVSI PWTHKVGN
47 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
48 MPLSYQHFRKLLLLDE--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
49 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
50 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
51 MPLSYQHFRKLLLLDDGTEA GPLEEELPRLADADLNRRVA EDNLGNLNVSI PWTHKVGN
52 MPLSYQHFRKLLLLDDGTEA GPLEEELPRLADADLNRRVA EDNLGNLNVSI PWTHKVGN
53 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
54 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
55 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
56 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
57 MPLSYQHFRKLLLLDD--EA GPLEEELPRLADEGLNRRVA EDNLGNLNVSI PWTHKVGN
58 MPLSYQHFRKLLLLHD--EA GPLEEELPRLPDQGLNRRVA EDNLGNLNVSI PWTHKVGN
158 MPLSYQHFRKLLLLddGTEA GPLEEELPRLaDegLnrrVA EDNLGNLNVSI PWTHKVGN

61
39 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
40 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
41 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
42 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
43 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
44 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PK
45 FTGLYSSTVPVFNPEWQTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PK
46 FTGLYSSTVPVFNPEWQTPS FPKIHLQEDI INRCQQFVGP LTVNEKRRLKL IMPARFY PT
47 FTGLYSSTVPVFNPEWQTPS FPDHHLQEDI VDRCKQFVGP LTVNENRRLKL IMPARFY PN
48 FTGLYSSTVPVFNPEWQTPS FPDHHLQEDI VDRCKQFVGP LTVNENRRLKL IMPARFY PN
49 FTGLYSSTVPVFNPEWQTPS FPDHHLQEDI INKCKQFVGP LTVNEKRRLKL IMPARFY PN
50 FTGLYSSTVPVFNPEWQTPS FPDHHLQEDI INRCQQFVGP LTVNENRRLKL IMPARFY PN
51 FTGLYSSTVPVFNPEWQTPS FPKIHLQEDI INRCQQFVGP LTVNEKRRLKL IMPARFY PT
52 FTGLYSSTVPVFNPEWQTPS FPKIHLQEDI INRCQQFVGP LTVNEKRRLKL IMPARFY PT
53 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI INRCQQYVGP LTVNEKRRLKL IMPARFY PN
54 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI IKKCEQFVGP LTVNEKRRLQL IMPARFY PK
55 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI IKKCEQFVGP LTVNEKRRLQL IMPARFY PN
56 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI IKKCEQFVGP LTVNEKRRLQL IMPARFY PK
57 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI IKKCEQFVGP LTVNEKRRLQL IMPARFY PK
58 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI IKKCEQFVGP LTVNEKRRLQL IMPARFY PN
158 FTGLYSSTVPVFNPDWKTTPS FPHIHLQEDI INRCQQFVGP LTVNEKRRLKL IMPARFY PN

Fig. 4 (Sheet 1 of 7)

5/15

121

39 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 40 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 41 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 42 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 43 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 44 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 45 LTKYLPLDKGIKPYYPEHAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 46 HTKYLPLDKGIKPYYPDQVV NHYFQTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 47 VTKYLPLDKGIKPYYPEHV NHYFQTRHYLHTLWKAGILY KRESTHSASFCGSPYSWEQD
 48 VTKYLPLDKGIKPYYPEYVV NHYFQTRHYLHTLWKAGILY KRESTRSASFCGSPYSWEQD
 49 VTKYLPLDKGIKPYYPEHV NHYFQTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 50 VTKYLPLDKGIKPYYPEHV NHYFQTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQD
 51 HTKYLPLDKGIKPYYPDQVV NHYFQTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 52 HTKYLPLDKGIKPYYPDQVV NHYFQTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 53 LTKYLPLDKGIKPYYPEYAV NHYFKTRHYLHTLWKAGILY KRETTRSASFCGSPYSWEQE
 54 VTKYLPLDKGIKPYYPEHLV NHYFQTRHYLHTLWKAGILY KRETTHSASFCGSPYSWEQD
 55 VTKYLPLDKGIKPYYPEYLV NHYFQTRHYLHTLWKAGILY KRETTHSASFCGSPYSWEQE
 56 VTKYLPLDKGIKPYYPEHLV NHYFQTRHYLHTLWKAGILY KRETTHSASFCGSPYSWEQD
 57 VTKYLPLDKGIKPYYPEHLV NHYFQTRHYLHTLWKAGILY KRETTHSASFCGSPYSWEQD
 58 VTKYLPLDKGIKPYYPEHLV NHYFQTRHYLHTLWKAGILY KRETTHSASFCGSPYSWEQE
 150 VTKYLPLDKGIKPYYPEh V NHYFQTRHYLHTLWKAGILY KRETtTsASFCGSPYSWEQe

151

39 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSMARGKSG
 40 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSLARGKSG
 41 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSLARGNQG
 42 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCIRSQ KQSRLGLQPQOGSLARGKSG
 43 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ TQSRLGLQPQOGSLARGKSG
 44 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSLARGKSG
 45 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSLARGKSG
 46 LQH-----SQRHGDESFC SQPSGILSRSSVVGPCIRSQ NKSRLGLQPHQGFLASSQPG
 47 LQHGRLVFQTSKRHGDKSFC PQSPGILPRSSVVGPCIQSQ RKSRLGPQPTQGQLAGRPOG
 48 LQHGRLVFQTSKRHGDKSFC PQSSGILPRSSVVGPCIQSQ RKSRLGPQPEQGQLAGRQOG
 49 LQHGRLVLOTSTRHGDKSFR PQSSGILSRSPVGPCIQSQ RQSRLGPQPTQGQLAGLQOG
 50 LQHGRLVLOTSTRHGDKSFR PQSSGILSRSPVGPCIQSQ RQSRLGPQPTQGQLAGLQOG
 51 LQHGRLVIKTSQRHGDESFC SQPSGILSRSSVVGPCIRSQ KQSRLGLQPHQGFLASSQPG
 52 LQHGRLVIKTSQRHGDESFC SQSSGILSRSSVVGPCIRSQ KQSRLGLQPRQGR LASSQPS
 53 LQHGRLVFQTSTRHGDESFC SQSSGILSRSPVGPCVRSQ KQSRLGLQPQOGSLARGKSG
 54 LQH-----AESFH QQSSGILSRPPVGSSLQSKH RKSRLGLQSQQGH LARRQOG
 55 LQH-----AESFH QQSSGILSRPPVGSSLQSKH RKSRLGLQSQQGH LARRQOG
 56 LQH-----AESFH QQSSGILSRPPVGSSLQSKH RKSRLGLQSQQGH LARRQOG
 57 LQH-----AESIH QQSSGILSRPPVGSSLQSKH RKSRLGLQSQQGH LARRQOG
 58 LQH-----AESFH QQSSGILSRPPVGSSLQSKH RKSRLGLQSQQGH LARRQOG
 150 LQHGRLVfqtStRHGdesfc sQssGILsRspVGpc.rSql .qSRLGLQpQOG.lAr.qqg

Fig. 4 (Sheet 2 of 7)

SUBSTITUTE SHEET (RULE 26)

6/15

39 RSGSIRARVHPTTRRSFGVE PSGSGHIDNSASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 40 RSGSIRARVHPTTRRSFGVE PSGSGHIDNSASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 41 RSGRLRARVHPTTRRSFGVE PSGSGHIDNSASSASSCFHQ SAVRKTAYSHLSTSKROSS
 42 RSGSIWARVHSTTRRSFGVE PSGSGHIDNSASEASSCLYQ SAVRKTAYSHLSTSKROSS
 43 RSGSIRARVHPTTRRSFGVE PAGSGRIDNRASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 44 RSGSIRARVPPTTRRSFGVE PSGSGHIDNRASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 45 RSGSIRARVPPTTRRSFGVE PSGSGHIDNRASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 46 RSGSIRARAHPPSTRYFGVE PSGSGHIDHSVNNSSCLHQ SAVRKAAYSHLSTSKROSS
 47 GSGSIRARIHPSPWGTVGVE PSGSGHTHCASSSSSCLHQ SAVRTAAYSPISTSKGHSS
 48 GSGSIRARVHPSPWGTVGVE PSGSGPTHNCASSSSSCLHQ SAVRKAAYSLIPTSKGHSS
 49 GSGSIRAGIHSTPWGTVGVE PSSSGHTHNCANSSSSCLHQ SAVRKEAYSPVSTSKRHSS
 50 GSGSIRAGIHSTPWGTVGVE PSSSGHTHNCANSSSSCLHQ SAVRKEAYSPVSTSKRHSS
 51 RSGSIRARVHPSTRRCFGVE PSGSGHVDPSVNNSSCLRQ SAVRKAAYSHLSTSKROSS
 52 RSGSIRAKAHPPSTRYFGVE PSGSGHIDHSVNNSSCLHQ SAVRKAAYSHLSTSKROSS
 53 RSGSIWSRVHPTTRRPFGE PSGSGHIDNTASSTSSCLHQ SAVRKTAYSHLSTSKROSS
 54 RWSIRAGFHPTARRPFGVE PSGSGHTTNFASKASCLHQ SPVRKAAYPAVSTFEKHSS
 55 RWSIRAGIHPTTRRPFGE PSGSGHTRIVASKASCLYQ SPVRKAAYPAVSTFEKHSS
 56 RWSIRAGFHPTARRPFGVE PSGSGHTTNFASKASCLHQ SPVRKAAYPSVSTFEKHSS
 57 WWSIRAGTHPTABRPFGVE PSGSGHTTHRASKASCLYQ SPDRKATYPSVSTFEKHSS
 58 RWSIRAGFHPTARRSFGVE PSGSGHTTYRASKASCLYQ SPVRKAAYPSVSTFEKHSS
 59 rsgs irarv hpttrr. fGVE PsgSGhIdn. assssSCLhQ SavRkaaYshlStskrqSSS

301

39 GHAVEFHNI PPSSARSQSEG PIFSCWWLQFRNSKPCSDYC LTHIVNLLDWDGPCTEHGEH
 40 GHAVELHNI PPSSARSQSEG PILSCWWLQFRNSKPCSDYC LTHIVNLLDWDGPCTEHGEH
 41 GHAVELHNI PPSSARSQSEG PIFSCWWLQFRNSKPCSDYC LTHIVNLLDWDGPCTEHGEH
 42 GHAVELHNI PPSCARSQSEG PISSCWWLQFRNSEPCSDYC LTHIVNLLDWDGPCTEHGEH
 43 GHAVELHNI PPSSARSQSEG PILSCWWLQFRNSKPCSDYC LTHIVNLLDWDGPCTEHGEH
 44 GHAVELHHISPSPARSQSEG PIFSSWWLQFRNSKPCCDYC LTHIVNLLDWDGPCTEHGEH
 45 GHAVELHHISPSPARSQSEG SVSSCWWLQFRNSKPCSEYC LSHLVNLRDWDGPCDDEHGEH
 46 GHAVEFHCLAPSSAGSQSQ SVLSCWWLQFRNSKPCSEYC LSHIVNLIEDWGPCAHEHGEH
 47 GHAVELHHFPPNSSRSRSQ SVLSCWWLQFRNSEPCSEYC LCHIVNLIEDWGPCTEHGEH
 48 GHAVELHHFPPNSSRSRSQ SVLSCWWLQFRNSKPCSEHC LFHIVNLIEDWGPCAHEHGEH
 49 GNAVELHHVPPNSSRSRSQ SVLSCWWLQFRNSKPCSEHC LSHLVNLRDRGPCDEHGEH
 50 GHAVELHHVPPNSSRSRSQ SVFSCWWLQFRNSKPCSEYC LSHLVNLRDWDGPCDEHGEH
 51 GHAVEFHCLPPSSARSQSQ PIFSCWWLQFRNSKPCSDYC LTHIVNLLDWDGPCTEHGEH
 52 GHAVEFHCLPPSSAGSQSQ PVFPCWWLQFRNSKPCSDYC LSLIVNLLDWDGPCAHEHGEH
 53 GHAVELHNI PPSSARSQSEG PVFPCWWLQFRNSKPCSDYC LSHIVNLLDWDGPCAHEHGEH
 54 GHAVEFHNLPPNSARSQSER PVFPCWWLQFRNSKPCSDYC LSLIVNLLDWDGPCAHEHGEH
 55 GHAVELHNLPPNSARSQSER PIFPCWWLQFRNSKPCSDYC LSLIVNLLDWDGPCDEYGEH
 56 GHAVELHNLPPNSARSQSER PVFPCWWLQFRNSKPCSDYC LSLIVNLRDWDGPCTEHGEH
 57 GRAVELHNLPPNSARSQSER PIFPCWWLQFRNSKPCSDYC LSLIVNLLDWDGPCDEYGEH
 58 GHAVELHNLPPNSARSQSER PVFPCWWLQFRNSKPCSDYC LSLIVNLRDWDGPCTEHGEH
 59 GhAVELHn. pPnsarsqSeg pviScWWLQFRNSkPCsdyc L.hivNlledWgPctehGEH

Fig. 4 (Shear 2 of 7)

7/15

361

39 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 40 NIRIPRTPARVTGGVFLVDK NPHNTTESTLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 41 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 42 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 43 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 44 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 45 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 46 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG ITRVSWPKFAVPNLQSLTNL
 47 RIRTPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG NTRVSWPKFAVPNLQSLTNL
 48 RIRTPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG NTRVSWPKFAVPNLQSLTNL
 49 RIRTPRTPARVTGGVFLVDK NPHNTSESRLVVD FSQFSRG NTRVSWPKFAVPNLQSLTNL
 50 RIRTPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG NTRVSWPKFAVPNLQSLTNL
 51 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG ITRVSWPKFAVPNLQSLTNL
 52 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG ISRVSWPKFAVPNLQSLTNL
 53 NIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG STHVSWPKFAVPNLQSLTNL
 54 HIRIPRTPSRVTTGGVFLVDK NPHNTAESRLVVD FSQFSRG NYRVSWPKFAVPNLQSLTNL
 55 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG NYRVSWPKFAVPNLQSLTNL
 56 HIRIPRTPSRVTTGGVFLVDK NPHNTAESRLVVD FSQFSRG NYRVSWPKFAVPNLQSLTNL
 57 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG NYRVSWPKFAVPNLQSLTNL
 58 HIRIPRTPARVTGGVFLVDK NPHNTAESRLVVD FSQFSRG NYRVSWPKFAVPNLQSLTNL
 158 HIRIPRTPARVTGGVFLVDK NPHNTTESRLVVD FSQFSRG ITRVSWPKFAVPNLQSLTNL

421

39 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINHGHGAMQDL
 40 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV VCLSSTSKNINYQHGTMOQL
 41 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINYQHGTMOQL
 42 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINYQHGTMOQL
 43 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINYQHGTMOQL
 44 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINYQHGTMOQL
 45 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINHGHGAMQDL
 46 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLIGSSGLSRYV ARLSSNSRIINNQHGTMOQL
 47 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMOQL
 48 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHRTMOQL
 49 LSSDLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHRTMOQL
 50 LSSDLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHRTMOQL
 51 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLIGSSGLSRYV ARLSSNSRIINNQHRTMOQL
 52 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLIGSSGLSRYV ARLSSNSRIINNQHRTMOQL
 53 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLPRYV ARLSSTSRNINYQHGTMOQL
 54 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMPDL
 55 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMOQL
 56 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMOQL
 57 LSSNLSWLSLDVSAAGFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMPNL
 58 LSSNLSWLSLDVSAAFYHLP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMOQL
 158 LSSNLSWLSLDVSAAFYHIP LHPAAMPHLLVGSSGLSRYV ARLSSNSRIINNQHGTMOQL

Fig. 4 (Sheet 4 of 7)

SEQUENCE SHEET (PAGE 2)

8/15

481

39 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 40 HDSCSRNLYVSLFLLYKTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 41 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 42 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 43 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG GGLSPFLLAQFTSAICSVVR
 44 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG GGLSPFLLAQFTSAICSVVR
 45 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG GGLSPFLLAQFTSAICSVVR
 46 HDSCSRQL
 47 HNCSRNLYVSLMMLLYKTYG WKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 48 HNCSRNLYVSLMMLLYKTYG WKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 49 HDSCSRNLYVSLMMLLYKTYG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 50 HDSCSRNLYVSLMMLLYKTYG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 51 HDSCSRQLVSLMMLLYKTYG WKLHLYSHPIVLGFRKIPMG VGLSPFLLAQFTSAICSVVR
 52 HDSCSRQLVSLMMLLYKTYG WKLHLYSHPIVLGFRKIPMG VGLSPFLLAQFTSAICSVVR
 53 HDSCSRNLYVSLLLLYKTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 54 HDYCSRNLYVSLLLLYQTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 55 HDSCSRNLYVSLLLLYQTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 56 HDSCSRNLYVSLLLLYQTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 57 HDSCSRQLVSLMMLLYQTFG WKLHLYSHPIVLGFRKIPMG VGLSPFLLAQFTSAICSVVR
 58 HDSCSRNLYVSLLLLYQTFG RKLHLYSHPIILGFRKIPMG VGLSPFLLAQFTSAICSVVR
 158 Hdscsrnlyvslillyktfg rklhlyshpiilgfrkipmg vglspfllaqftsaicsvvr

541

39 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 40 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 41 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 42 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 43 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 44 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 45 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 46 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 47 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 48 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 49 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 50 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 51 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 52 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 53 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 54 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 55 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 56 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 57 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 58 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY
 158 RAFFHCLAFSYMDDVVLGAK SVQHLESLEFSTITNFFLSLG IHLNPNKTKRWGYSLNFMGY

Fig. 4 (Sheet 5 of 7)

SUBSTITUTE SHEET (RULE 26)

9/15

Fig. 4 (Sheet 6 of 7)

601
39 VIGSWGTLPOEHIVLKLKQC FRKL PVNSPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
40 VIGCWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
41 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
42 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
43 VIGCWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
44 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
45 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
47 VIGSWGTLPOEHIVQKIKMW FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
48 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
49 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
50 VIGSWGTLPOEHIVLKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
51 VIGSWGTLPODHIVQKIKHC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
52 IIGSWGTLPODHIVQKIKHC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
53 VIGSWGTLPOEHIVQKLKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
54 VIGCYGSLPOEHIIQKIKEC FRKL PINRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
55 VIGCYGSLPODHIIQKIKEC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
56 VIGCYGSLPOEHIIQKIKEC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
57 VIGCYGSLPOEHIIQKIKEC FRKV PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY
58 IIGSWGTLPODHIVQKIKEC FRKL PVNRPIDWKVQRIVG LLGFAAPFTQCGYPALMPLY
158 VIGSWGTLPOEHIVQKIKQC FRKL PVNRPIDWKVCQRIVG LLGFAAPFTQCGYPALMPLY

661
39 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
40 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
41 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
42 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
43 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
44 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVAR-RTALCQVF ADATPTGWGLAIGHRRMRGT
45 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVAR-RTALCQVF ADATPTGWGLAIGHRRMRGT
47 ACIQAKQAFTFSPTYKAFLS KOYNTLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
48 ACIQAKQAFTFSPTYKAFLT KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
49 ACIQAKQAFTFSPTYKAFLN KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
50 ACIQAKQAFTFSPTYKAFLT KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
51 ACIQAKQAFTFSPTYKAFLS KOYMNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
52 ACIQAKQAFTFSPTYKAFLS KOYMNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
53 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRSGLCQVF ADATPTGWGLAIGHRRMRGT
54 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
55 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
56 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
57 ACIOFKQAFTFSPTYKAFLC KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
58 ACIQAKQAFTFSPTYKAFLS KOYMNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT
158 ACIQSKQAFTFSPTYKAFLC KOYLNLYPVARQRPGLCQVF ADATPTGWGLAIGHRRMRGT

721
39 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
40 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
41 FVVPLPIHTAELLAACFARD RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
42 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
43 LNLCSRSLRNS
44 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTYFV
45 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTYFV
47 FVSPLPIHTAELLAACFARS RSGANLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
48 FVSPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
49 FVSPLPIHTVELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
50 FVSPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
51 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
52 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
53 FVAPLPIHTAELLAACFARS RSGAKLIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
54 FSAPLPIHTAELLAACFARS RSGANIIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
55 FLARLPIHTAELLAACFARS RSGANIIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
56 FSAPLPIHTAELLAACFARS RSGANIIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV
57 FSAPLPIHTAELLAACFARS RSGANIIGTDNSVVLRSKYT SFPWLLGCAANWILRGTSFV

SUBSTITUTE SHEET (RULE 26)

10/15

781

39 YVPSALNPADDPSRGRGLGY RPLLLLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 40 YVPSALNPADDPSRGRGLGY RPLLHLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 41 YVPSALNPADDPSRGRGLGY RPLLSLPFQPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 42 YVPSALNPADDPSRGRGLGY RPLLHLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 44 YVPSALNPADDPSRGRGLGY RPLLHLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 45 YVPSALNPADDPSRGRGLGY RPLLHLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 47 YVPSALNPADDPSRGRGLGY RPLLHLPFRPTTGRTSLYAV SPSVPSHLPDRVHFASPLHV
 48 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 49 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 50 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 51 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 52 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 53 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 54 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 55 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 56 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 57 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 58 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV
 158 YVPSALNPADDPSRGRGLGY RPLLRLPYRPTTGRTSLYAD SPSVPSHLPDRVHFASPLHV

841

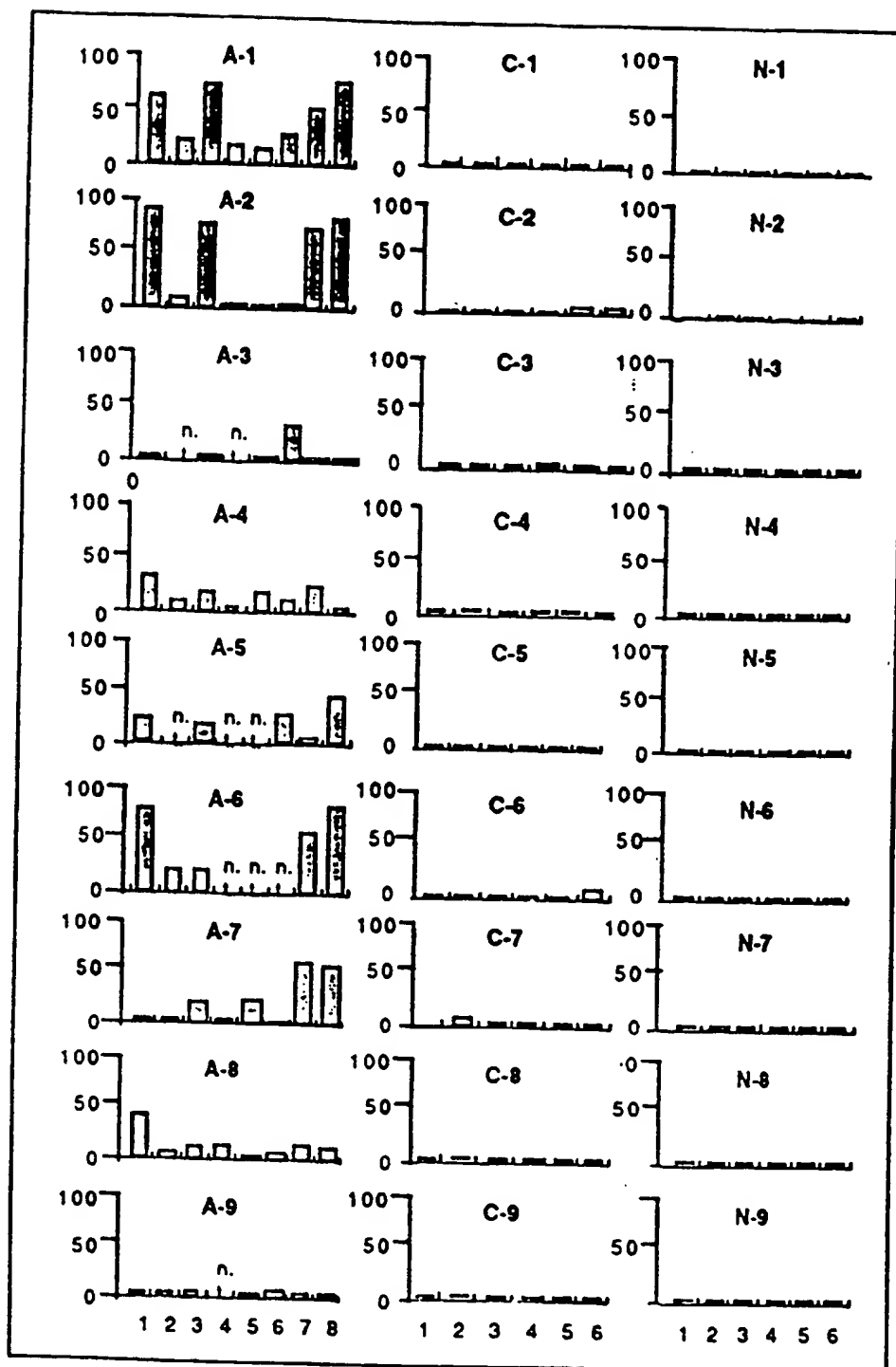
39 AWRPP
 40 AWRPP
 41 AWRPP
 42 AWRPP
 44 AWRPP
 45 AWRPP
 47 AWRPP
 48 AWRPP
 49 AWRPP
 50 AWRPP
 51 AWRPP
 52 AWRPP
 53 AWRPP
 54 AWRPP
 55 AWRPP
 56 AWRPP
 57 AWRPP
 58 AWRPP
 158 AWRPP

Fig. 4 (Sheet 7 of 7)

SIRSTITITE SHEET (R111 F 26)

11/15

% SPECIFIC CYTOTOXICITY



PEPTIDE SPECIFICITY

1 POL455-463

4 POL655-663

6 POL816-824

2 POL551-559

5 POL773-782

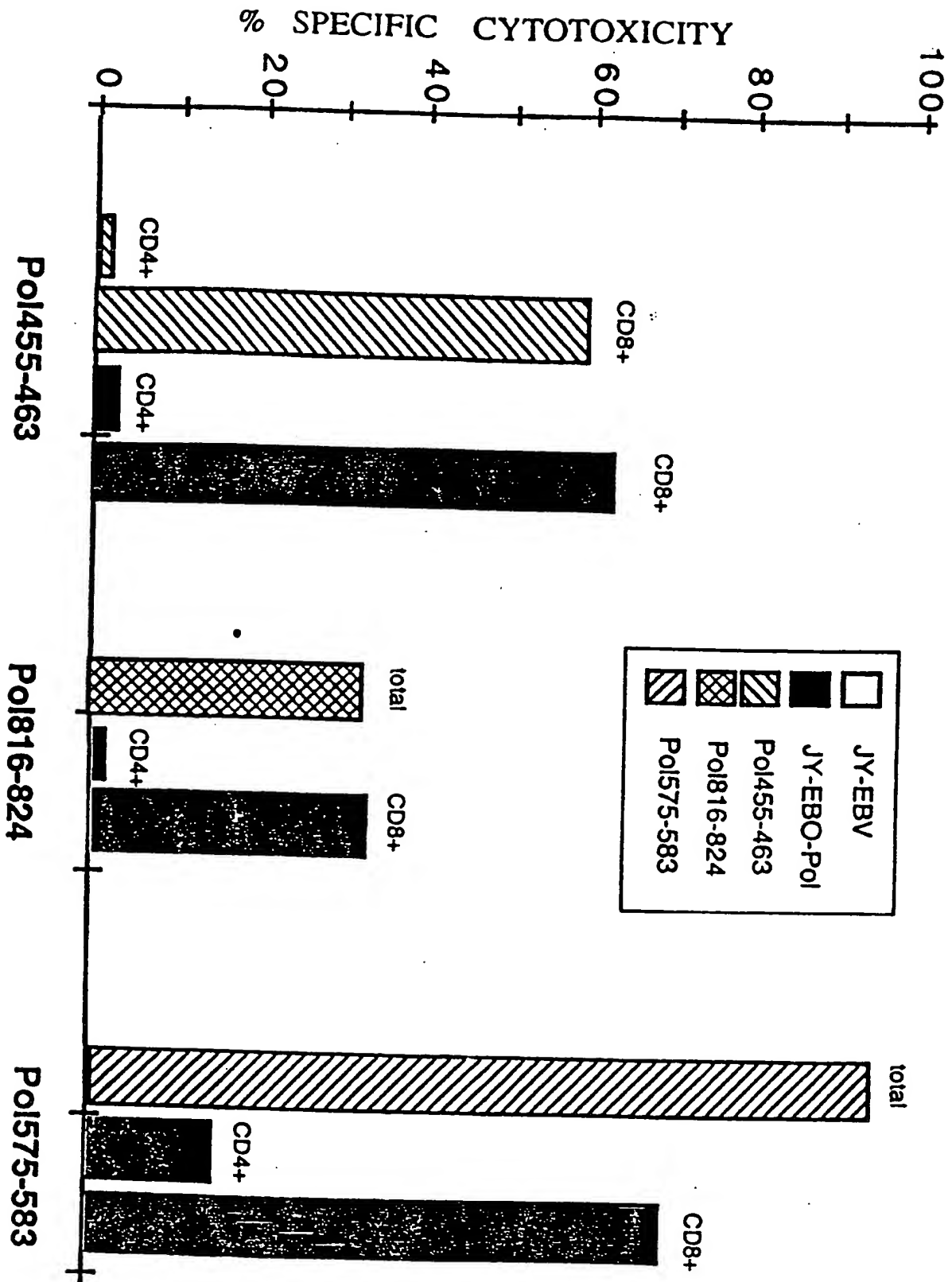
7 CORE18-27

3 POL575-583

8 ENV335-343

Fig. 5
 SUBSTITUTE SHEET (RULE 26)

12/15



SUBSTITUTE SHEET (RULE 26)

Fig. 6

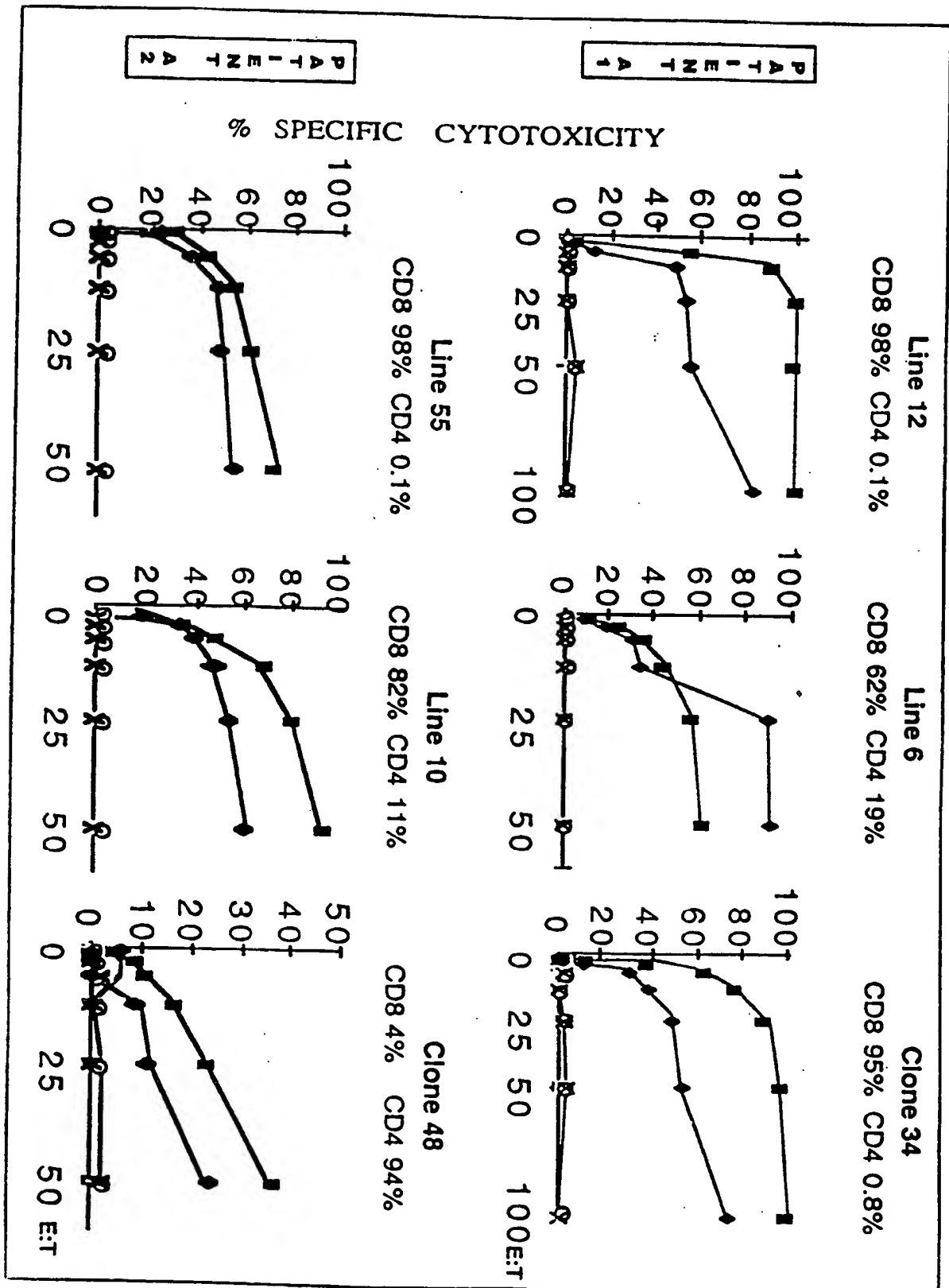


Fig. 7

14/15

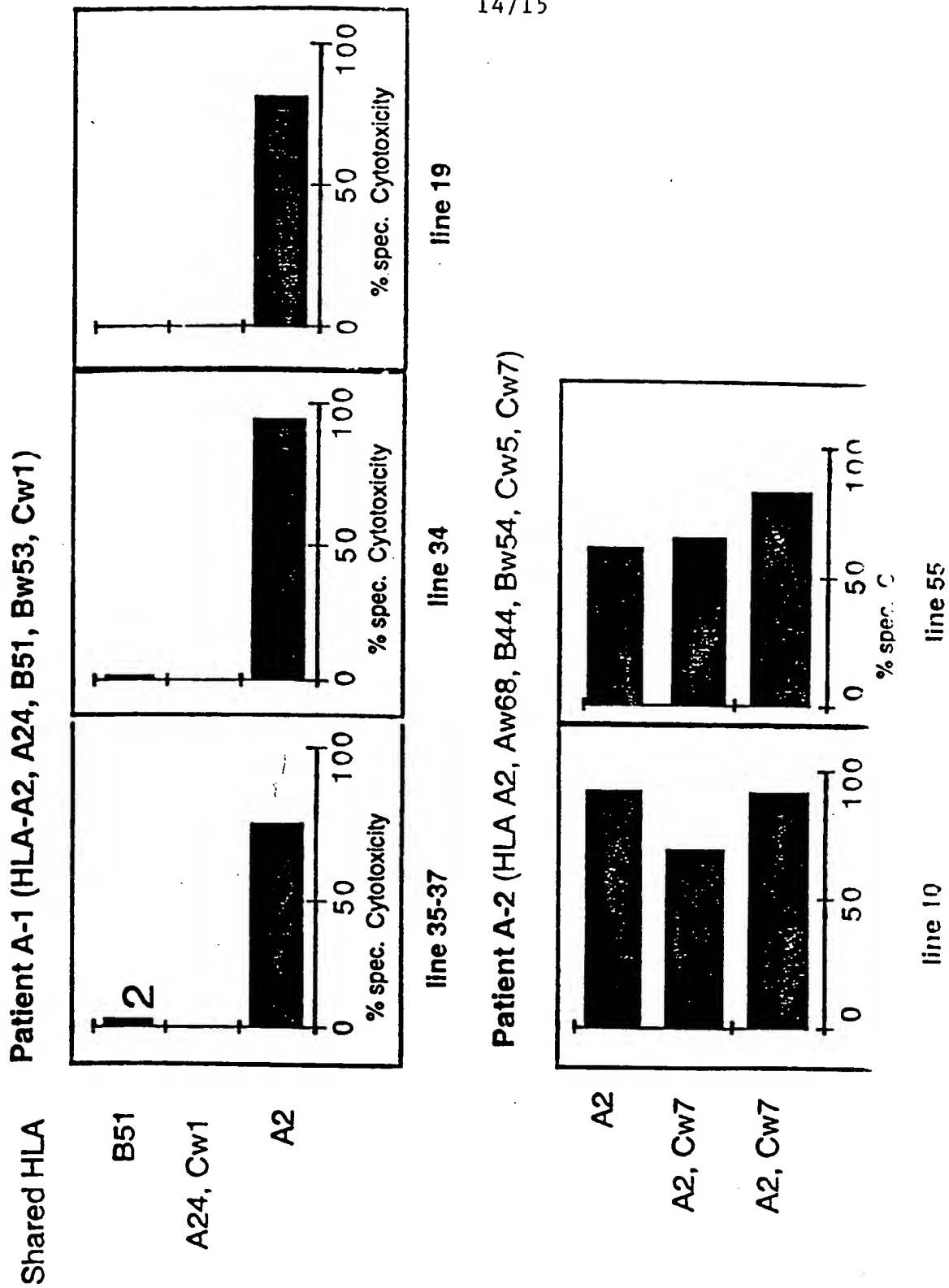


FIG. 8

15/15

FIG. 9A

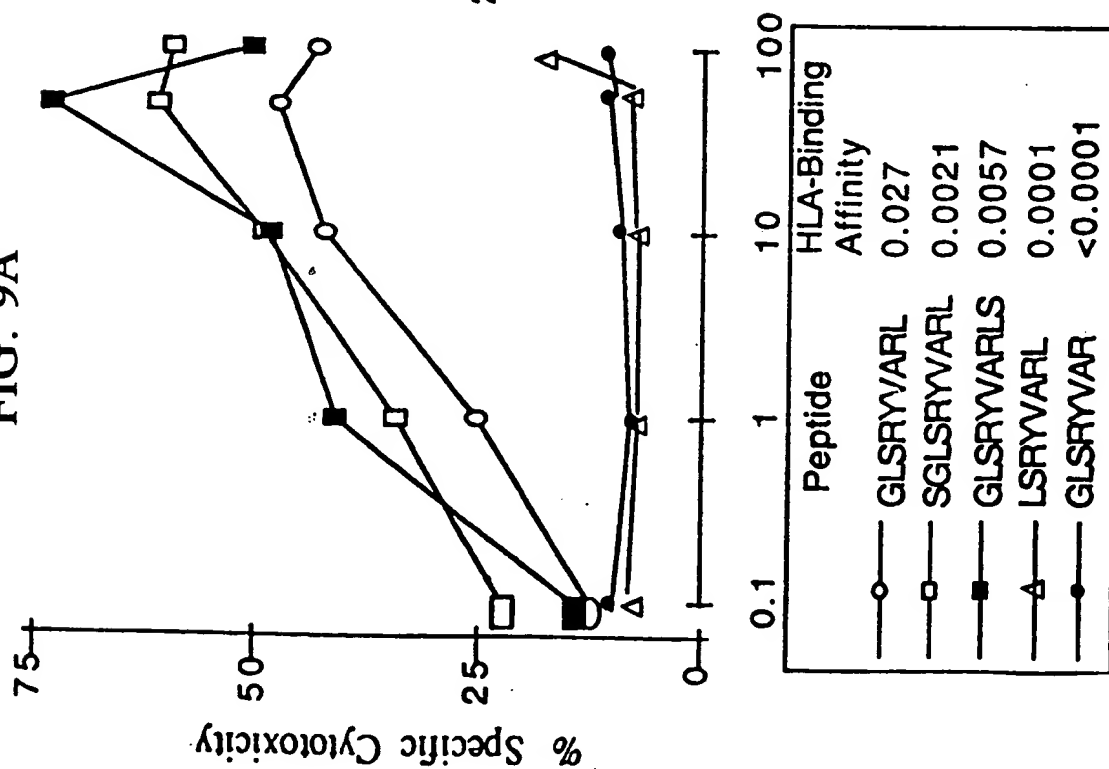
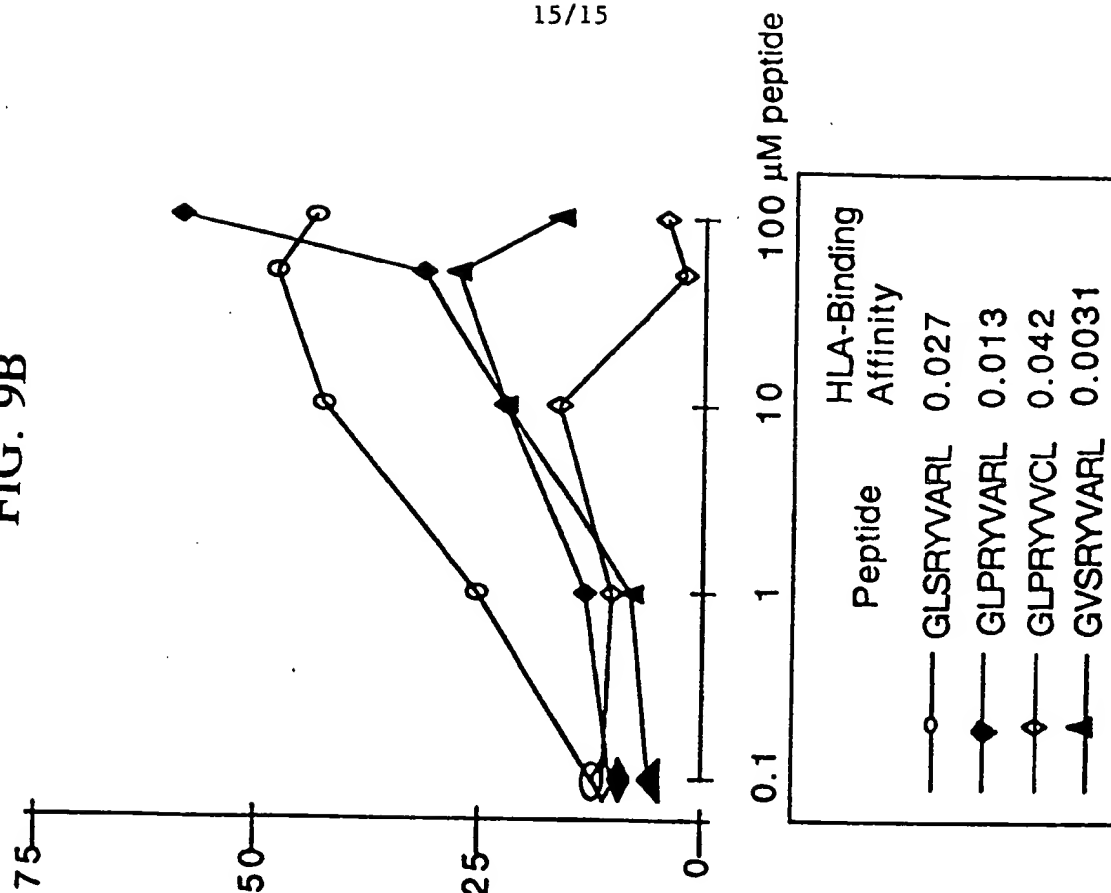


FIG. 9B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08685

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89, 450; 435/69.1, 240.2; 512/2, 12; 530/300, 403; 536/22.1, 23.1, 23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, BIOSIS


search terms: cytotoxic t lymphocyte#, ctl, hepatitis b virus, hbv, hbpol, polymerase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. EXP. MED., Volume 167, issued June 1988, Carbone et al., "Induction of Cytotoxic T Lymphocytes by Primary In Vitro Stimulation with Peptides", pages 1767-1779, see entire document.	1-16
Y	JOURNAL OF VIROLOGY, Volume 62, Number 12, issued December 1988, Mack et al., "Hepatitis B Virus Particles Contain a Polypeptide Encoded by the Largest Open Reading Frame: A Putative Reverse Transcriptase", pages 4786-4790, see entire document.	1-16
Y	NATURE, Volume 281, issued 25 October 1979, Galibert et al., "Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in E. coli", pages 646-650, see entire document.	1-16

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 01 OCTOBER 1994	Date of mailing of the international search report 10 NOV 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Hyosuk Kim  Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08685

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Microbial Pathogenesis, Volume 6, issued 1989, F. Chisari et al., "Mini Review. Hepatitis B Virus Structure and Biology", pages 311-325, see entire document.	1-16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/08685

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 7/46, 39/12, 37/22, 53/00; A01N 37/08; C07H 17/00, 19/00, 21/00; C12P 21/06; C12N 5/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/89, 450; 435/69.1, 240.2; 512/2, 12; 530/300, 403; 536/22.1, 23.1, 23.72